Gunilla Nevander

EPILEPTIC BRAIN DAMAGE

An experimental study using a recovery model of status epilepticus

Lund 1985

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1. Nevander, G., Ingvar, M., Auer, R., Siesjö, B.K. (1984) Irreversible neuronal damage after short periods of status epilepticus. Acta Physiol Scand 120:155-157 51

2. Nevander, G., Ingvar, M., Auer, R., Siesjö, B.K. (1985) Status epilepticus in well-oxygenated rats causes neuronal necrosis. Ann Neurol (in press) 55

3. Auer, R., Ingvar, M., Nevander, G, Olsson, Y., Siesjö, B.K. (1985) The development of hypermetabolic infarction in the substantia nigra in status epilepticus: 2. Morphology (in manuscript) 65

4. Nevander, G., Ingvar, M., Lindvall, O. (1985) Mechanisms of epileptic brain damage: Evidence for a protective role of the noradrenergic locus coeruleus system (submitted for publication). 107

5. Folbergrová, J., Ingvar, M., Nevander, G., Siesjö, B.K. (1985) Cerebral metabolic changes during and following fluorothyl-induced seizures in ventilated rats. J. Neurochem 44:1419-1426 121

6. Ingvar, M., Nevander, G. (1985) Local cerebral glucose utilization and blood flow in the rat during and following flurothyl-induced status epilepticus (in manuscript) 129

7. Siesjö, B.K., von Hanwehr, R., Nergelius, G., Nevander, G., Ingvar, M. (1985) Extra- and intracellular pH in the brain during seizures and in the recovery period following arrest of seizure activity. J Cereb Blood Flow Metabol 5:47-57 153

INTRODUCTION

An epileptic seizure can be defined as the sudden onset of recurrent, paroxysmal, synchronous electrical discharges of neurons, either focal or generalized to the brain as a whole. For a long time, it has been known that seizures may result in brain damage. It is also established that some brain areas are more vulnerable than others. The cause of the brain damage and the selective vulnerability has remained unknown, although substantial efforts have been made to clarify the underlying mechanisms.

This thesis attempts to elucidate some aspects on brain damage seen after generalized seizures. It is based on experiments performed on rats with flurothyl (hexafluorodietyl ether) as the seizure inducing agent. This convulsant gas has the ability to elicit generalized seizures when added to the inspired air (Krantz 1963). Its mode of action is believed to be through opening of sodium channels in the cell membrane (Woodbury 1980). Previously, it has been used to induce short-lasting seizures (Sacktor et al. 1966, Howse et al. 1974), but not for long-term status epilepticus (SE). After short-lasting seizures, exhalation of the gas results in seizure termination. Following seizures of longer duration, the seizures can be terminated by withdrawal of flurothyl supply, and by the administration of a single dose of thiopental (Nevander et al. 1984, 1985). Our novel model for flurothyl-induced status epilepticus allows seizures to be sustained for extended periods, since the gas is retained in a rebreathing system. In contrast to other general convulsants commonly used in experimental conditions (e.g. bicuculline and pentylenetetrazol), flurothyl allows long-term survival of the animals after seizure termination. This has offered us the unique possibility

to characterize physiological and neurochemical events in the postical period, as well as during seizures, and to study the development and establishment of brain damage.

BACKGROUND

1. Histopathological changes due to seizures

Post-mortem examination of brains in chronic epileptic patients have revealed brain injury of varying degrees (for review, see Meldrum and Corsellis 1984). In reports including large series of epileptic patients, such a damage has been described in more than 50 per cent of the patients. Some brain areas seem more susceptible to develop lesions than others. The regions most often reported to be affected are the hippocampus and the cerebellum. In the hippocampus, the Sommer sector (CA_1) and the endfolium (CA_4) are the areas most regularly damaged. In the cerebellum, the Purkinje cells are the most vulnerable cellular elements. Other vulnerable structures are the cerebral neocortex, especially the intermediate layers, the thalamus and the amygdala. In all affected regions, the damage consists of neuronal loss and gliosis, sometimes with scarring and atrophy.

Similar lesions are seen after generalized seizures induced with a variety of convulsants in animal experiments (see Meldrum and Corsellis 1984). However, it is shown that if systemic complications like hypotension and hyperpyrexia are avoided, then the cerebellum is spared. Although controversial, generalized seizures shorter than 90 min are in general not considered to result in brain damage (see Delgado-Escueta et al. 1983). Previous experimental studies on histopathological outcome after sustained generalized seizures have been carried out with animal models that do not allow long-term survival of the animals after seizure termination. This is because the

convulsants used (like e.g. bicuculline) give rise to deletorious systemic complications which necessitates sacrification of the animals within a few hours of recovery following seizure arrest. Another problem has been to terminate the seizure activity without creating a harmful influence on the general status of the animals. Söderfeldt et al. (1983) used large doses of diazepam to arrest bicuculline seizures, which led to marked hypotension in the recovery period. Recent research in the ischemia field have revealed that the final cell damage "matures" over hours and days, neuronal necrosis being preceded by a free interval (Ito et al. 1975, Kirino et al. 1982, Pulsinelli et al. 1982). The study by Söderfeldt et al. (1983) indicated that a major part of the cellular changes observed in the neocortex and in the hippocampus at 1 or 2 hours of bicuculline-induced status epilepticus can be reversible in the immediate recovery period. However, due to the lack of a suitable animal model, it has not been possible to study histopathological outcome after prolonged recovery periods, and to address the question of whether there is "maturation" of final epileptic brain damage as of the ischemic injury.

2. Normal cerebral metabolism and circulation

In the adult brain, and in the absence of ketosis, energy production is exclusively based on metabolism of glucose. Metabolism of glucose via the aerobic pathway yields 38 moles of ATP for each mole of glucose consumed. The energy produced by oxidation of glucose is thus stored in the molecule of ATP, and cellular work is then performed at the expense of ATP. When utilized, ATP is degraded to ADP and orthophosphate (P_i). Breakdown of ATP followed by increase in ADP leads, by the adenylate kinase reaction, to accumulation of AMP.

Degradation of AMP leads, among other things, to production of adenosine. The balance of production and utilization of energy can be expressed as an equation according to Atkinson (1968):

$$EC = \frac{[ATP] + 0.5[ADP]}{[ATP] + [ADP] + [AMP]}$$

The energy charge (EC) is, under ordinary conditions, regulated by efficient mechanisms to around 0.94.

When lack of oxygen occurs, energy can be produced by the anaerobic metabolism of glucose. However, glycolysis yields only 2 moles of ATP for each mole of glucose consumed. Additional energy is available in the form of phosphocreatine (PCr), which can phosphorylate ADP via the creatine kinase (CrK) reaction:

$$PCr + ADP + H^+ \overset{CrK}{\rightleftarrows} Cr + ATP$$

Provided uninterrupted energy utilization is at hand, the brain tissue stores of high-energy phosphate groups (ATP and PCr) will suffice only for very limited time periods (around 20 sec, see Siesjö 1978). This underlines the necessity for a continous oxidative metabolism.

Except for being delivered by the blood, glucose is available as glycogen, which is a storage form of glucose. However, tissue stores of glucose and glycogen are small. Glycogen breakdown is assumed to be triggered by several different stimuli (see Siesjö 1978). There are two interconvertible forms of glycogen phosphorylase, the enzyme responsible for glycogen degradation. Energy failure may induce glycogenolysis by activation of phosphorylase b, since the action of

this enzyme is inhibited by ATP but activated by AMP and P_i. In stressful situations, catecholamines are released. These, i.e. adrenaline and noradrenaline, are believed to activate adenylate cyclase, forming cyclic AMP (cAMP) from ATP (Bloom 1975, Iversen 1977). Cyclic AMP in turn converts phosphorylase b to phosphorylase a, which induces glycogenolysis. Enhanced cellular activity leads to influx of Ca^{2+} into the cells. Ca^{2+} can convert phosphorylase b to the a form, and thereby induce glycogenolysis. Except for inducing glycogenolysis, there is evidence that cAMP serves as a second messenger for NA to mediate inhibitory actions on central synapses (Bloom 1975, Stone et al. 1975)

Depolarization is started by opening of Na^+-channels in the post-synaptic cell membrane. This opening is mediated by excitatory transmitters, e.g. glutamate, aspartate and acetylcholine. The transmitter release is triggered by influx of Ca^{2+} into pre-synaptic nerve terminals (Blaustein et al. 1978). Inhibition is mediated by inhibitory transmitters e.g. GABA (γ-aminobutyric acid) and noradrenaline (NA), and implies opening of K^+ and/or Cl^- gates. Restoration following depolarisation is at the expense of ATP, through Na^+/K^+ activation of membranebound ATPase. This involves extrusion of Na^+ from the cell and influx of K^+ into the cell. Ca^{2+} seems to be extruded by an antiport system, exchanging Ca^{2+} for Na^+, and probably also by Ca^{2+} pumps utilizing ATP in a more direct way (see Baker and Reuter 1975).

It follows that the maintainance of energy homeostasis in the brain is exclusively dependent of a continuous supply of oxygen and glucose via arterial blood, since available energy stores are very small. Cerebral blood flow (CBF) is regulated by variations of two factors, namely

perfusion pressure and cerebrovascular resistance. Normally, CBF is autoregulated, i.e. constant if the perfusion pressure is varied in between limits of 60-160 mmHg (see Edvinsson and MacKenzie 1977). The mechanisms regulating the coupling of CBF to cerebral metabolism in a purposeful way are not known. Several factors reflecting cellular activity have been proposed, among them extracellular increase of K^+, H^+ and adenosine (for review, see Siesjö and Ingvar 1983).

3. Cerebral changes during seizures

The most intense cerebral activity known is elicited by seizures. Hence metabolic rate is markedly increased during status epilepticus. A large number of investigations have been performed concerning cerebral metabolic rate and blood flow during seizures. This presentation concentrates on changes seen during generalized seizures. Studies on metabolic rate in whole brain or cortex with pentylenetetrazol or bicuculline as the seizure-inducing agent have revealed two-to threefold increases in glucose- and oxygen consumption (Plum et al. 1968, Borgström et al. 1976, Meldrum and Nilsson 1976). Blennow et al. (1978, 1979) showed that metabolic rate was elevated for 2 hours of bicuculline-induced seizures, provided glucose and oxygen supplies were upheld and discontinuation of the supplies did not lead to disappearance of the seizure discharge. In general, the increased metabolic rate during seizures is matched by an increase in CBF. The CBF increase connected to seizure activity was noted already 1890 by Roy and Sherrington, and have been confirmed later by numerous investigators, showing an increase in whole brain and regional CBF of between 1.5 and 9 times control values (Meyer et al. 1966, Plum et al. 1968, Howse et al. 1974, Meldrum and Nilsson 1976). Due to the intense vasodilation, autoregulation is suspended during seizures (Plum et al.

1968), and arterial blood pressure therefore profoundly influences the CBF. This might explain the large variability in results reported concerning CBF-increase during generalized seizures.

As shown for the whole brain, metabolic rate and CBF are increased in a seemingly matched way during seizures. On the local level, though, the coupling between metabolic rate and blood flow is more variable. In a recent study Ingvar et al. (1983), using autoradiographic techniques for measurements of local CBF (l-CBF) and local metabolic rate for glucose (l-CMRgl) during bicuculline-induced seizures, revealed that at least in some structures a mismatch develops between local CBF and local CMRgl. Thus, structures like the cortical regions, the hippocampus and the amygdala, known to be susceptible to develop brain damage after seizures, showed sustained increases in metabolic rates but developed relative underperfusion during seizures extended for 2 hours. Another finding of interest was that, following an initial increase in metabolic rate, the substantia nigra and the globus pallidus subsequently developed very low CMRgl values during 2 hours of seizure activity, while the l-CBF still was increased to values above controls.

Several studies of cerebral metabolic events have shown that seizures, even when prolonged for 2 hours, cause only minimal deterioration of the phosphorylation state of the tissue adenine nucleotide pool provided tissue oxygenation is optimal, and no systemic complications arise in the form of severe hypoglycemia, hyperthermia and hypotension (Duffy et al. 1975, Chapman et al. 1977, Blennow et al. 1977, 1979, Folbergrová et al. 1981, Siesjö et al. 1982, Ingvar et al. 1984). Perturbation of energy state is more pronounced in areas such as the neocortex and the hippocampus, assumed to be susceptible to epileptic

insults than in the cerebellum, a resistant area (Folbergrová et al. 1981, Siesjö et al. 1982). Under optimal conditions, the main metabolic effects are restricted to reduced glycogen concentrations by glycogenolysis, increased concentrations of lactate and pyruvate by stimulation of glycolysis, raised concentrations of cyclic nucleotides, reduction of the cytoplasmic NADH/NAD^+ system, and accumulation of free fatty acids (FFAs). All these changes are assumed to reflect accelerated cerebral metabolic rate. If moderate hypoxia with pO_2 values around 45-50 mmHg is at hand, the major metabolic alteration is an accentuation of lactic acidosis (Blennow et al. 1977).

Cerebral extracellular pH (pH_e) changes in acidotic direction during short-lasting seizures (Jasper and Erickson 1941, Wang and Sonnenschein 1955, Caspers and Speckman 1972, Astrup et al. 1978, Heuser 1978, 1981). Also intracellular pH (pH_i) has been reported to decrease during seizures of short duration (Howse et al. 1974, Duffy et al. 1975, Chapman et al. 1977). No information exists on pH_e or pH_i changes during sustained seizures.

4. Cerebral changes in the postical period

Due to lack of suitable recovery models, allowing long-term survival of experimental animals, information about circulatory and neurochemical events in the postictal period is limited. Several investigators, making their observation on patients, have reported a depression of CBF in the recovery period after seizures (Grant et al. 1947, Kety et al. 1948, Meyer et al. 1966, Magistretti et al. 1983). However, the magnitude and temporal extent of this blood flow

reduction have remained unknown, and there are no reports on postictal metabolic rate for glucose. Thus we lack information on the metabolic rate/blood flow couple in the postictal period.

Previous studies on cerebral metabolic state in the postictal period are undertaken after relatively shortlasting seizures, and also the recovery periods were rather short (Howse et al. 1974, Folbergrová et al. 1974, 1977). To summarize the findings, energy state, as reflected in ATP and PCr content of the tissue, and cAMP regained normal values within 5 minutes of recovery, whereas lactate and glycogen normalized more slowly. No comprehensive report exists on postictal cerebral pH_e and pH_i.

OBJECTIVES OF THE PRESENT STUDY

The purposes of this thesis were as follows:

1. To develop a model for generalized seizures in rats, which allows long-term survival of the animals after seizure termination.

2. To characterize this model concerning cerebral metabolic state, blood flow and glucose utilization during seizures. A comparison of the results obtained with data from acute experiments using general convulsants as bicuculline and pentylenetetrazol, would determine if the model is generally valid for generalized seizures in rats.

3. To delineate changes in intra- and extracellular pH during seizures and in the recovery period

4. To study postictal events regarding cerebral metabolic state, blood flow and glucose utilization, since a delineation of these could possibly provide insights into the mechanisms responsible for induction of brain damage.

5. To evaluate the localization and the extent of brain damage after status epilepticus of varying duration.

6. To study the evolution of the final brain damage.

7. To investigate if lesions of the inhibitory noradrenergic locus coeruleus system can influence the extent of epileptic brain damage incurred in certain brain areas.

1. Animals and operative techniques

All experiments were performed on adult male Wistar rats of a S.P.F. strain (Møllegaards Breeding Center, Copenhagen). The animals had free access to pellet food and tap water prior to the experiments. Except for when sterotactic lesions of the locus coeruleus were performed, anesthesia was induced with halothane in N_2O/O_2 (70/30). The animals were intubated (in longterm recovery experiments) or tracheotomized, artificially ventilated and paralyzed with suxamethonium chloride. The ventilator was adjusted to obtain an arterial pO_2 of above 90 mmHg and pCO_2 to between 30 and 40 mmHg. The femoral arteries, alternatively the tail artery, were cannulated to allow continuous blood pressure recording, blood sampling for analysis of blood gases, pH and glucose, and for withdrawal of blood prior to seizure induction. In the experiments designed for blood flow measurements, one brachial artery was cannulated to obtain blood samples (Dahlgren et al. 1981a). Catheters were also inserted in the femoral or the tail veins for infusions of drugs, isotope and blood. A bipolar EEG was recorded from needle electrodes or gold-plated screws inserted in the skull bone. Body temperature was measured rectally and kept close to 37°C by external heating.

Animals in experiments used for analysis of cerebral metabolites or total CO_2 content were prepared for freezing *in situ* by placement of a plastic funnel over the skull bone. In animals used for measurement of arterial and cerebrovenous pCO_2, a burr hole was drilled over the superior sagittal sinus for sampling of cerebral venous blood. When

preparing for measurement of extracellular pH (pH_e) or tissue impedance, we placed a craniotomy (about 3 x 3 mm) over the frontoparietal cortex to allow insertment of the electrodes.

Lesion techniques

The lesions of the noradrenergic system were performed under barbiturate anesthesia with the animals placed in a stereotactic instrument. Bilateral lesions of the ascending noradrenergic fibres from the nucleus locus coeruleus were made at the level of the caudal mesencephalon by injections of 6-hydroxydopamine (Dahlgren et al. 1981b). Further experiments were performed 13-22 days later. The efficiency of the lesions was checked by analysis of the noradrenaline (NA) content in the frontal poles.

2. Instigation, maintenance and termination of seizures

Seizures were induced by adding flurothyl to a rebreathing system connected to the inlet of the respirator and containing a CO_2 absorber. Prior to addition of the convulsant, arterial blood was withdrawn and phentolamine was infused i.v. in order to curtail the ictal blood pressure increase at the start of the seizure period. If necessary to maintain arterial blood pressure above 100 mmHg, the shed blood was reinfused and donor blood was given. Additional boluses of flurothyl was given as required to sustain a burst-suppression EEG-pattern. After the desired seizure period the flurothyl supply was discontinued, and the animal connected to a normal open ventilator system. This was as a rule sufficient to terminate seizure discharge after short seizure periods ($\leq$ 30 min). In animals subjected to more prolonged status epilepticus, it was in general necessary to arrest the seizures with a short-acting barbiturate (thiopental i.v.).

During the seizures and early recovery period blood or plasma glucose, blood gases and pH were monitored repeatedly. Arterial pO_2 was kept above 90 mmHg (except in animals deliberately made hypoxic) and pCO_2 between 30-40 mmHg by adjustment of the respirator. Animals subjected to long-term survival were extubated when spontaneous breathing movements returned. Their general status and weight were then checked repeatedly during the following days.

3. Tissue sampling and analytical techniques

a) Blood gases and pH: Measurements of arterial pO_2, pCO_2 and pH were performed on anaerobically obtained samples of 150-200 µl, using microelectrodes operating at 37°C. The values obtained were corrected for body temperature.

b) Cerebral metabolites: At the termination of the experiment, the brains were frozen *in situ* with liquid nitrogen (Pontén et al. 1973), chiselled out and stored at -80°C for later dissection at -22°C. For measurement of labile phosphates, carbohydrate substrates and cyclic AMP the tissue samples were extracted with HCl-methanol followed by perchloric acid. Following centrifugation and neutralization of the extracts, enzymatic-fluorometric techniques were used to determine labile phosphates and carbohydrate substrates (see Folbergrová et al. 1981). Similar techniques were used to measure glucose, lactate and pyruvate in arterial blood. A similar extraction technique was used to determine cAMP with a protein binding technique. Plasma glucose was measured by use of a Beckman automatic glucose analyzer.

c) Total CO_2: The total CO_2 content of the frontoparietal cortex was measured by microdiffusion techniques (Pontén and Siesjö 1964, see also Siesjö et al. 1972).

d) Noradrenaline: NA-content of tissue samples, taken from the frontal poles immediately before perfusion fixation, was analyzed as described by Schmidt et al. (1982), using a radioenzymatic method derived from Da Prada and Zürcher (1976).

4. Local cerebral blood flow and local cerebral glucose utilization. Both l-CBF and l-CMRgl were measured with autoradiographic techniques. Local CBF was determined according to Sakurada et al. (1978), using ^{14}C-iodoantipyrine as the diffusible tracer. The isotope was infused i.v. over a period of 20 or 45 sec depending on the expected flow rate. During isotope infusion, timed arterial samples were taken repeatedly from one brachial artery to assess ^{14}C-activity with β-scintillation counting. Simultaneously with the last arterial sample, the animal was decapitated using a guillotine.

We measured local cerebral glucose utilization using the technique described by Sokoloff et al. (1977). ^{14}C-deoxyglucose was infused i.v. over a perid of 30 sec. During a 45 min period, starting with the infusion, timed arterial blood samples were taken for determination of glucose content and also for ^{14}C-activity by β-scintillation counting. At 45 min the animals were decapitated.

For both l-CBF and l-CMRgl determinations the further procedures were as follows: The brains were removed from the skulls, immediately frozen in isopenthane at -50°C to -70°C, and stored at -80°C. Sectioning of the brains in 20 µm thick slices was done using a Leitz sledge microtome. The sections obtained were dried on a hot plate and then exposed to an X-ray film for one week together with a set of calibrated ^{14}C standards. The density of the brain structures was then evaluated using a densitometer with an aperture of 1 mm (Macbeth TD

501). Each structure was measured bilaterally on three different sections, and the mean values were used for calculations of l-CBF and l-CMRgl.

5. Histological procedures

Perfusion fixation was performed on tracheotomized or re-intubated rats, artificially ventilated with 1 % halothane in N_2O/O_2 (70/30). A cannula was introduced into the ascending aorta via the left ventricle, and after isotonic saline flushing, perfusion fixation with phosphate-buffered aldehydes was carried out. For light microscopy, brains were fixed with 4 % formaldehyde, dehydrated, embedded in paraffin, sagitally sectioned at 8 µm and double stained with acid fuchsin/cresyl violet. For electron microscopy, and for light microscopy with tissue embedding in soft plastic, brains were fixed with 3 % glutaraldehyde. Following this, they were dehydrated, embedded in a soft methacrylate polymer, sectioned and stained with acid fuchsin/cresyl violet or toluidine blue (light microscopy). For electron microscopy, sections were embedded in Epon 812, stained with uranyl acetate and lead citrate, and examined on a JEOL JEM 100C electron microscope.

6. Quantification of brain damage

With the acid fuchsin stain, necrotic neurons were seen in the light microscope as bright red masses of cytoplasm, often containing deep red staining nuclei. These acidophilic neurons were considered as necrotic, since they have been shown to disappear from tissue sections with survival periods longer than one week (Auer et al. 1984). Direct visual counting of acidophilic neurons was performed at a magnification of 320 x, using a laboratory cell counter. The distribution of necrotic neurons was noted in sections throughout the

whole brain, and to obtain quantitative results, acidophilic neurons in the cerebral cortex, hippocampus and caudate nucleus were counted. Sectioning intervals were adapted to obtain identical portions of these regions.

7. Measurement of extracellular pH

Changes in pH_e was measured by use of double-barrelled, liquid ion-exchange microelectrodes with tip diameters of 1-3 μm inserted into the cerebral cortex. Construction of the electrodes were as described by Kraig et al. (1983), and Mutch and Hansen (1984). Prior to seizure induction, the electrodes were calibrated at 37°C in phosphate buffers (pH 6.0-7.8), and electrode performance was tested by measurement of pH_e-changes during short-lasting hypercapnia.

8. Measurements of tissue impedance

Cerebral cortical impedance was measured by using a four-electrode technique (see Pelligrino et al., 1981). Electrode tips were inserted to a depth of ~ 500-700 μm into the cortex.

9. Calculations

The energy charge of the adenylate pool was calculated according to Atkinson (1968).

Changes in the cytoplasmic $NADH/NAD^+$ ratio were calculated from the lactate dehydrogenase equilibrium:

$$\frac{[NADH]}{[NAD^+]} = \frac{[Lact]}{[Pyr]} \times \frac{K}{[H^+]}$$

as described previously by Chapman et al. (1977).

Changes in intracellular pH were derived from the creatine kinase equilibrium or by the CO_2 method as formerly described by Siesjö et al. (1972).

Tissue impedance changes were calculated by use of the Maxwell and Raleigh equations (see Pelligrino et al. 1981).

Local CBF was calculated according to Sakurada et al. (1978), and local CMRgl as described by Sokoloff et al. (1977).

Statistical differences were evaluated by use of analysis of variance (ANOVA), followed by Newman-Keul's or Dunnett's test to establish differences between groups. When normal distribution could not be assumed, the Mann-Whitney U-test was used.

RESULTS AND DISCUSSION

1. Physiological and EEG changes

In all animals, seizure induction was followed by an initial increase in arterial blood pressure. However, due to prior withdrawal of blood and administration of phentolamine, the blood pressure peak in general did not exceed 160-170 mmHg. By infusion of blood, it was possible to avoid hypotension during seizures and in the recovery period, even after administration of thiopental (cf. Söderfeldt et al. 1983). Seizures were accompanied by a slight systemic acidosis with pH values usually not falling below 7.20, even in animals with SE sustained for two hours. In the early recovery period, arterial pH was close to control already 5 min after seizure arrest. Moderate hypoxia (arterial pO_2 40-50 mmHg) during 20 min SE did not aggravate the systemic

acidosis. In long-term survivals, the general status of the animals during the recovery period, following extubation, was related to the length of the seizure period. The longer the seizure period, the slower was the recovery. Extubation was possible after 1-3 hours. During the two hours following extubation, the rats displayed sparse spontaneous movements and reduced reaction to different stimuli. Gradually, recovery followed, and within 2-3 days the majority of the animals appeared normal. Convulsions were not seen in the recovery period. All animals were able to drink and eat within 24 hours after completion of the experiment. In spite of this, they lost weight in proportion to the length of the seizure period.

Flurothyl seizures evoked a similar EEG-pattern (see paper 5) as was previously described for SE induced by bicuculline (see e.g. Chapman et al. 1977, Ingvar et al. 1983) or pentylenetetrazol (Ingvar et al. 1984). Within 30 sec after administration of flurothyl, an epileptic pattern of changes was recorded on the EEG. After an initial period (up to 5 min) of spikes and waves, a burst-suppression pattern appeared, which then prevailed for the rest of the seizure period. The EEG-recordings were similar for normoxic and hypoxic animals. Seizure termination, either simply by discontinuation of the flurothyl supply, or by administration of thiopental, was followed by EEG-depression. EEG-activity subsequently returned and, following a seizure period of 20 min, the EEG pattern was normalized at 45 min after seizure arrest.

Paper 1

In this article, the newly-developed flurothyl model for long-term survival after generalized seizures is described, and preliminary findings of histopathological alterations after seizures are reported.

Ventilated animals were subjected to seizure periods ranging from 15 to 90 min under optimal physiological conditions, and sacrified after one week of survival. After perfusion fixation, light microscopic examination of brain sections with counting of acidophilic neurons was performed. Two novel findings of importance were made. First, it was clearly demonstrated that unequivocal brain damage was incurred already after a seizure period as short as 30 min. Earlier, it was generally assumed that generalized seizures of less than 90 min duration does not result in brain damage, although this has been controversial (see Delgado-Escueta et al. 1983). Second, this early lesion was localized to the pars reticulata of the substantia nigra (SNPR) and to the globus pallidus (GP). In these regions, infarction was seen after seizure periods of 30 min or longer. Following seizures sustained for 60 min or more, moderate selective neuronal necrosis was seen in the cerebral cortex, the hippocampal CA1 and CA4 regions, and in thalamic nuclei. However, due to the small number of experiments performed in this preliminary study, the time-related establishment of lesions and the extent of the damage could not be described in detail.

Paper 2

It has been debated for a long time if brain damage incurred after status epilepticus is caused by the seizure discharge *per se*, or if systemic complications like arterial hypotension might induce the lesions. However, several earlier studies have demonstrated that even when hypoxia, hypotension, hypoglycemia and hyperthermia are avoided,

animals develop brain damage after seizures (e.g. Meldrum et al. 1973, McIntyre et al. 1982). Studies of histopathological outcome after generalized seizures have been impeded by lack of a suitable recovery model, allowing long-term survival. The importance of this is obvious, since it has recently been shown that most of the alterations seen acutely after bicuculline-induced seizures can be reversible in the immediate recovery period (Söderfeldt et al. 1983), and that after ischemia, the final cell damage "matures" over a prolonged period (Ito et al. 1975, Kirino et al. 1982, Pulsinelli et al. 1982).

This study was designed to assess the extent and the localization of the final brain damage after flurothyl-induced status epilepticus of varying duration. The question of whether there is "maturation" of epileptic brain injury is addressed in a subsequent publication (paper 3). Animals were subjected to 15, 30, 45, 60, 90 and 120 min of generalized seizures under optimal tissue oxygenation. Hypotension, hyperthermia and hypoglycemia were thus avoided. Seizures were arrested simply by withdrawal of flurothyl supplies, or by thiopental i.v. One week thereafter, brains were perfusion fixed, subserially sectioned and examined under light microscopy. Counting of acidophilic neurons in parietal cortex, hippocampus and caudoputamen was performed.

Results obtained showed that two patterns of distribution of damage existed, one confined to certain nuclei and cortical laminae, and one with relationship to the cerebral ventricles and aqueduct. No brain damage was seen after 15 min of seizure activity. After 30 min or more, lesions were seen in the pars reticulata of the substantia nigra (SNPR) and in the globus pallidus (GP). The nigral lesions consisted of overt infarction. In the GP, spongiosis of the neuropil and

neuronal necrosis were seen, and in some animals, there were central infarcts. After 45 min of seizures, moderate neocortical damage was seen, progressively increasing after more prolonged seizure periods, but never extensive. Cortical neuronal necrosis was most pronounced in layers 3 and 4 over the superolateral convexity of the hemispheres, and extended from the frontal to the occipital cortex. Seizures prolonged for 60 min or more induced selective neuronal necrosis in the hippocampal CA1 and CA4 pyramidal cells, as well as in the amygdala and in thalamic nuclei, chiefly in the ventral posterior nuclei. Some acidophilic Purkinje cells were seen in the cerebellum after 90-120 min of seizures, and the periventricular pattern of neuronal necrosis was clearly discernible first after 90 min of status epilepticus. To summarize, extensive damage with infarction was only seen in the SNPR, and in the GP. Elsewhere, the damage consisted of moderate selective neuronal necrosis. Also, the longer the seizure periods, the more structures were damaged, and the more extensive was the local damage.

The results described confirm the preliminary findings of paper 1: In ventilated, well-oxygenated rats, where systemic complication were avoided, obvious and irreversible brain damage was seen in the SNPR and GP already after 30 min of generalized seizures. Lesions of the SNPR have previously been seen in acute experiments on pentylenetetrazol-induced seizures (Howse et al. 1983). Re-examination of slides showed that similar SNPR infarcts were also found in our own histopathological material from acute bicuculline experiments. Thus, we conclude that the SNPR lesion is not "flurothyl specific". The pattern of distribution of brain damage after flurothyl seizures seems similar as for bicuculline-induced seizures (Blennow et al. 1978) and as for pentylenetetrazol-induced ones (Ingvar et al. 1984). This is a

tentative conclusion, though, since data for extended recovery periods are lacking for the two latter convulsants. The periventricular pattern of neuronal necrosis observed in our flurothyl material resembles the distribution pattern of hypoglycemic damage (Auer et al. 1984).

The SNPR has recently been reported to mediate protection from convulsive activity (Iadorala and Gale 1982). Our data supports that the SNPR may be crucial in that sense, since lesion development in the SNPR is timed with appearance of self-sustained seizures. Animals with large infarcts in the SNPR required thiopental to terminate seizure activity.

Paper 3

This study was undertaken to delineate the development of the final brain damage seen after flurothyl seizures. The experiments were performed on ventilated, well-oxygenated rats with avoidance of systemic complications. The SNPR and GP were examined under light- and electronmicroscopy after 20, 35, 45, and 60 min of seizure activity, and after 1, 3, 6, 24 hours and one week of recovery following 60 min of status epilepticus. Sixty min of flurothyl seizures was previously known regularly to be followed by infarction in the SNPR, and also regularly by lesions in the GP (paper 2).

Light microscopical examination of the SNPR revealed that at 20 min of status epilepticus there was a pallor of the neuropil and appearance of dark neurons with microvacuolation of the cytoplasm. The latter finding also appeared in otherwise normal neurons. The changes mentioned above all remained during the more prolonged seizure periods examined. One week after the insult, macrophage infiltration was seen

in the periphery of the necrotic central core. Also in the GP, there was a pallor of the neuropil during status epilepticus. During recovery, mild damage was characterized by microvacuolation of the neuropil, with simultaneous sparing of neurons. When slightly more damage was at hand, this was evident as macrophage infiltration into the gray matter and disappearance of neurons. When mild or moderate damage appeared, only the gray matter was affected. In severely damaged animals, both gray and white matter was affected, yielding a picture of pan-necrosis. In both SNPR and GP, infarct development started in the center of the nucleus.

Electron microscopy of the developing lesions in both SNPR and GP revealed a progressive series of alterations during seizures, beginning in the axons of the synaptic neuropil. Here, swollen mitochondria with fractured cristae was seen at 20 min of seizure activity. At 35 min of SE, dendrites showed similar alterations. At 60 min SE, axons and dendrites were indiscriminately damaged, showing swollen and ruptured mitochondria, often containing flocculent densities. At 35 min of seizure activity, cell membrane breaks were seen, leading to cavitation of the neuropil. In myelinated axons, resorption of the axons within intact myelin sheaths was seen. Membrane breaks of neuronal cell bodies and mitochondrial flocculent densities appeared at 45 min of SE. The same alterations, both indicative of cell necrosis, were seen in glial cells at 60 min of SE. Of the vascular elements, endothelial cells displayed only reactive changes and never necrotized, whereas pericytes that are located further away from the vascular lumen than endothelial cells, underwent necrosis.

Our results indicate that the infarcts seen in SNPR and GP after flurothyl induced seizurs are caused by a spreading process starting in the axons. Also, it is shown that the damage develops during the seizure period, and there is no "recovery" of affected structures or "maturation" of the damage as is seen after ischemia (Ito et al. 1975, Kirino et al. 1982, Pulsinelli et al. 1982). Flurothyl seizures have been shown to be followed by a pronounced increase in metabolic rate in the SNPR (paper 6). In a recent study (Ingvar et al. 1985) flurothyl seizures of 60 min were shown to be followed by metabolic failure in the SNPR with markedly reduced ATP-values, decreases of the adenylate pool and to an extreme local lactic acidosis with lactate values around 25 $\mu mol.g^{-1}$ in the SNPR. At 60 min of recovery following 60 min of seizures, the ATP-values and the EC were still decreased, and the lactate values above controls. The findings were interpreted as indicative of mitochondrial failure, this in turn suggested to be due to excessive Ca^{2+} influx into the axons caused by the intense and sustained neuronal stimulation during prolonged seizures. It was proposed that the massive Ca^{2+} influx could lead to overload of the Ca^{2+} sequestering mechanisms, thereby causing mitochondrial damage. Seizures are followed by an increased rate of glycolysis which leads to an enhanced lactate production (Chapman et al. 1977, Folbergrová et al. 1981, Ingvar et al. 1984, see also paper 5). If mitochondrial derangements exists, the lactate produced by glycolysis will accumulate and subsequently result in marked acidosis. Recently, it has been demonstrated by using DMO-autoradiography that a pronounced acidosis develops in the SNPR during flurothyl seizures (Siesjö et al. 1985). We suggest that the lactic acid is responsible for the spreading of the damage from the axons to the other affected cellular elements within the SNPR and the GP. Cerebral white matter drains the interstitial fluid of the brain (Klatzo et al. 1967, Brown and

Brierley 1972). The difference in extent of damage between the SNPR and the GP could thus be explained by the fact that the GP is richer in white matter than the SNPR, leading to more efficient drainage of lactic acid from the GP, and to less extensive lesions in this structure. We propose the term hypermetabolic infarction for the lesions developed.

Paper 4

This study was designed to investigate if lesions of the noradrenergic locus coeruleus (LC) system could modify neuronal necrosis seen after seizures. Previously, it was demonstrated that during status epilepticus, the locus coeruleus system is activated (Calderini et al. 1978) which is followed by an accumulation of cAMP (Harik et al. 1982, Ingvar et al. 1983, see also paper 5). Cyclic AMP is believed to serve as a second messenger for noradrenaline (NA) to mediate inhibition on central synapses (Bloom et al. 1975, Stone et al. 1975). The central noradrenergic system have been shown to possess seizure depressant properties (Libet et al. 1977, Mason and Corcoran 1979). However, no investigation is previously performed to see if the LC system also influences the extent of brain damage seen after seizures.

Two groups of ventilated and normoxic animals were studied. Systemic complications were avoided. In one, bilateral lesions of the ascending noradrenergic fibres from the LC was performed, whereas in the other, animals were sham-operated. Rats in both groups were then subjected to 60 min of flurothyl-induced SE, followed by survival for one week, and then perfusion-fixed. Assessment of NA-content in frontal poles of LC-lesioned animals revealed a reduction of NA-levels by 94 % when compared to sham animals. Counting of acidophilic neurons in the parietal cortex, hippocampus, caudoputamen and cerebellum demonstrated

that there was a significant increase in neuronal necrosis in the parietal cortex of NA-lesioned animals when compared to sham-operated rats (an average of 69 cells per section in NA-depleted rats, and an average of 22 cells per section in sham animals).

It has previously been suggested that selective neuronal necrosis after sustained seizures is induced by an excessive release of excitatory amino acids in the vulnerable regions (Collins and Olney 1982). The noradrenergic LC system, principally inhibitory, could counteract the detrimental neuronal hyperactivity, and thus limit the extent of neuronal necrosis. In a recent publication (Blomqvist et al. 1985), such a protective role for the LC system during ischemia was found, and the explanation suggested for the protection was as the above mentioned. A similar mechanism is proposed by us to be acting during flurothyl-seizures, changing the balance between excitatory and inhibitory stimulation towards a predominance of excitation in the LC-lesioned animals. In the neocortex, this is reflected as an increase in the number of necrotic neurons after 60 min of status epilepticus. We suggest that the LC lesions does not influence the extent of neuronal necrosis seen in the caudoputamen and cerebellum, since the caudoputamen contains very few noradrenergic fibres (Lindvall and Björklund 1978), and the lesion fails to sever the noradrenergic fibres to the cerebellum (unpublished observations). NA-levels in the hippocampus were not estimated in our study, but there is evidence that LC-lesions like those in our material sever most of the noradrenergic innervation in in this structure (Ingvar et al. 1983, Lindvall and Björklund 1978). The lack of influence of NA-lesions on hippocampal damage in our study could be explained if one assumes that the LC-system acts only as a modulator of the extent of brain damage. The number of necrotic neurons in the hippocampus,

averaging 4 per section in the sham group and 7 per section in the NA-depleted group, was probably too small to allow significant differences to appear between the NA-depleted and the sham-group. Longer seizure periods are followed by an increased number of necrotic neurons (see paper 2), and would probably reveal a protective NA-effect also in the hippocampus.

Paper 5

In this study, we wanted to characterize metabolic events in the neocortex and hippocampus of well-oxygenated or hypoxic rats during flurothyl seizures, and also in the recovery period following seizure arrest. By this, we wished to investigate if flurothyl seizures give rise to similar metabolic alterations as other commonly used general convulsants do (bicuculline and pentylenetetrazol). Also, we wanted to study the influence of moderate hypoxia (arterial pO_2 40-50 mmHg) upon metabolic state during the SE. Finally, we wished to delineate metabolic events during the immediate recovery period.

Two groups of rats were studied, one with normoxic and one with hypoxic animals. Complications like hyperthermia or arterial hypoglycemia and hypotension were avoided. All rats were subjected to 20 min of seizure-activity. Tissue was sampled from the fronto-parietal cortex and from the hippocampus at 5 and 20 min of seizure activity and 5, 15 and 45 min after seizure arrest.

In the cerebral cortex there were no statistically significant changes in adenine nucleotide concentrations during seizures neither in the normoxic, nor in the hypoxic group of animals. However, there was a tendency for ATP to decrease and for ADP and AMP to increase, the small changes recorded being most pronounced after 5 min seizure

activity in the hypoxic group. Energy charge was neither significantly altered in the normoxic, nor in the hypoxic group. Recovery values of adenine nucleotides were similar to those obtained during seizures and did not significantly differ from control. Energy charge values obtained during recovery were almost identical to those representing seizures sustained for 20 min. Results obtained from the hippocampus were similar to those described above for the neocortex. Phosphocreatine concentrations in both neocortex and hippocampus decreased significantly during seizures, progressively so in the hypoxic animals. Phosphocreatine values were normalized already after 5 min recovery in all groups studied. Our data are in agreement with those presented in earlier studies using bicuculline as the convulsant (e.g. Chapman et al. 1977), demonstrating that in ventilated and well-oxygenated animals there is only a minimal perturbation of the phosphorylation state of the tissue adenine nucleotide pool during sustained seizures. Previously, it has also been shown that the EC is not obviously deranged by moderate hypoxia (PaO_2 40-50 mmHg) during sustained bicuculline seizures (Blennow et al. 1977).

Both in neocortex and in hippocampus, cAMP increased significantly during seizures. The values obtained for the normoxic and hypoxic groups were almost identical in the respective structure. Cyclic AMP has previously been shown to increase during seizures in several studies (e.g. Folbergrová et al. 1981, Ingvar et al. 1984), probably reflecting, at least in part, activation of the central noradrenergic system (Calderini et al. 1978). In all groups studied, cAMP values were normalized already after 5 min of recovery.

A pronounced increase in lactate concentrations, reflecting enhanced glycolysis during seizures, was seen in the two structures analyzed. Values were identical in neocortex and hippocampus. In the normoxic group, the lactate accumulation reached maximal levels of around 10 $\mu mol.g^{-1}$ already at 5 min of seizure activity, whereas in the hypoxic group, a further increase in lactate contents to around 13-14 $\mu mol.g^{-1}$ was seen. However, compared to lactate data (Blennow et al. 1977) obtained from the neocortex after 2 hours of bicuculline induced status epilepticus with similar degree of hypoxia as in our study, the lactic acidosis seen at 20 min seizure activity in our material was moderate. Thus, one can conclude that marked acidosis (around 23 $\mu mol.g^{-1}$, Blennow et al. 1977) does not develop until seizures are sustained for more than 20 min, and that the lactate accumulation occurs gradually.

Enhanced glycogenolysis during seizures gave rise to markedly lowered glycogen concentrations in all animals studied.

During recovery, phosphocreatine and cAMP normalized within 5 min, whereas lactacidosis and depression of glycogen levels remained for a longer period, up to 45 min of recovery. The pattern of normalization was the same for both hypoxic and normoxic animals in the two structures analyzed.

Glucose concentrations in the neocortex were not changed significantly during seizures, but increased markedly during recovery in both the normoxic and in the hypoxic group. Also in the hippocampus, a postepileptic glucose accumulation was seen, here more accentuated in the hypoxic animals. In flurothyl seizures, as well as in seizures induced by bicuculline (Chapman et al. 1977, Folbergrová et al. 1981),

seizures were followed by a reduction of tissue-to-plasma glucose concentration ratios, presumably reflecting the increased glucose utilization in both regions (see paper 6). During recovery, the ratio was increased in the neocortex, in accordance with the pronounced depression of neocortical CMRgl values during recovery (paper 6). In the hippocampus the ratio as well as the CMRgl displayed values close to control in the postictal phase.

Intracellular pH was shown to decrease to acidotic values in neocortex during seizures in normoxic animals, whereas during the recovery period, after normalization of pH values during the first five postepileptic minutes, a delayed alkalosis appeared. Alkalosis has previously been described to occur in the recovery phase after ischemia (Mabe et al. 1983). The changes in intracellular pH, here calculated by use of the creatine kinase equilibrium, are confirmed by measurement of pH_i by a more direct method (paper 7).

In summary, flurothyl-induced seizures were shown to be followed by cerebral metabolic changes similar to those induced by bicuculline (Chapman et al. 1977, Blennow et al. 1977, 1979, Folbergrová et al. 1981) and pentylenetetrazol (Duffy et al. 1975, Ingvar et al. 1984). This indicates that the alterations seen are not specific for flurothyl. Further, after flurothyl seizures, cerebral metabolites normalize in a similar way as described in some previous studies with short-lasting seizures followed by short recovery periods (Howse et al. 1974, Folbergrová et al. 1974, 1977).

Paper 6

In this study, local cerebral glucose utilization (lCMRgl) and blood flow (lCBF) were measured during and following flurothyl seizures. Our main purpose was to characterize the local coupling between lCMRgl and lCBF during the immediate recovery period following seizure arrest, since knowledge on these matters might give insight into the mechanisms responsible for the depression of cerebral activity seen in the postical phase.

Both lCBF and lCMRgl were measured with autoradiographic techniques. Local cerebral blood flow was measured in rats at 20 min of continuous seizure activity, and at recovery 5 and 45 min following seizure termination. Local cerebral glucose utilization was studied in animals where the radioactive tracer was allowed to circulate between 5 and 45 min of seizure activity, and in two recovery group where the isotope was injected 5 and 45 min, respectively, after seizure arrest. Animals were ventilated and normoxic. Arterial hypoglycemia and complications like hypotension and hyperthermia were avoided.

Seizures lasting for 20 min induced a pronounced increase in lCBF in most of the structures measured. In the neocortical regions, the blood flow increase approximated 200 per cent of control. Some regions, e.g. the globus pallicus and substantia nigra, displayed an even more pronounced increase when calculated as per cent of control. The seizures also induced a marked enhancement in lCMRgl in a majority of the structures studied. In summary, flurothyl-induced seizures gave rise to similar alterations in lCBF and lCMRgl as bicuculline (Ingvar et al. 1983) and as previously is reported for lCBF during pentylenetetrazol seizures (Ingvar et al. 1984). Similar changes in

lCMRgl during pentylenetetrazol seizures as in flurothyl seizures also exist (preliminary observations). Also, the same local mismatch of CMRgl and CBF as in bicuculline-induced seizures (Ingvar et al. 1983) was apparent in this study. We therefore conclude, that seizures induced by either of the three convulsants is identical regarding local CMRgl and CBF.

In the recovery period 5 min after seizure termination, a general reduction of blood flow was seen, in accordance with the sparse data for postictal flow rates previously reported concerning epileptic patients (Grant et al. 1947, Meyer et al. 1966, Magistretti et al. 1983). The CBF-depression was most pronounced in the neocortical regions. At 45 min of recovery, the CBF-depression was still apparent in most structures measured, and prevailed pronouncedly reduced to values between 30 and 60 % of control in the neocortex. Also lCMRgl was, in general, reduced during recovery, although to a slightly lesser degree than lCBF. The local coupling between lCMRgl and lCBF was abolished at 5 min of recovery, but was essentially restored 45 min after seizure arrest. Since no studies on postictal local CMRgl and CBF existed previously, the presence, duration and magnitude of the local CMRgl-and CBF-depression are novel findings. In a previous communication (paper 5), we have shown that postictally, there is an increased tissue-to-plasma glucose concentration ratio in the neocortex. This suggests that there is no substrate deficiency and indicates that the reduction of blood flow is not limiting glucose availability, but that instead, there is a primary metabolic depression in the postictal period.

In paper 7 it is shown that the extracellular pH (pH_e) in the parietal cortex turns acidotic during flurothyl seizures sustained for 20 min, and that the acidosis remains almost unchanged during 45 min following seizure arrest. In this study, we have demonstrated a dramatic decrease of CBF in the parietal cortex at seizure arrest, from values around 200 per cent of control to values around 30 per cent of control. We conclude that pH_e is not the main regulator of CBF, as have previously been suggested (Lassen 1968, Howse et al. 1974).

Paper 7

This study was undertaken to estimate changes in intracellular pH (pH_i) and extracellular pH (pH_e) in the parietal cortex during flurothyl seizures and in the recovery period following seizure arrest. Previously, several investigators have recorded pH_e-changes during seizures using microelectrodes (Wang and Sonnenschein 1955, Howse et al. 1974, Astrup et al. 1978). However, there is not much information about the degree of acidosis developed during sustained seizures, and there is also some inconsistency in results obtained in those earlier studies. Sparse information is available about changes in pH_i during seizures. Some authors have reported an acidotic shift in pH_i as calculated from the creatine kinase equilibrium (Duffy et al. 1975) and by use of the CO_2 method (Howse et al. 1974). In one study in which bicuculline-seizures were sustained for 20 min, acidosis developed and the pH_i change approximated 0.2 units (Chapman et al. 1977). In a previous study we have calculated pH_i in the parietal cortex from the creatine kinase equilibrium during and following flurothyl seizures (paper 5). In this study, we wanted to correlate our results with those obtained when assessing pH_i from the

CO_2 method. We also wanted to delineate changes in pH_i and pH_e following arrest of prolonged seizures, since such data are failing due to the lack of a suitable recovery model.

In short, changes in pH_i were assessed at 5 and 20 min of seizure activity induced by flurothyl, and 5, 15 and 45 min after seizure arrest. Extracellular pH-changes was recorded continuously by use of microelectrodes during 5 or 20 min of seizures, and during a recovery phase up to 65 min. The pH changes were assessed in both normoxic and moderately hypoxic animals (arterial pO_2 40-50 mmHg). The rats were artificially ventilated, normothermic and normotensive, and hypoglycemia was avoided.

Results obtained in normoxic animals demonstrate that during seizures, the pH_i decreased within 5 min after seizure induction by 0.2 units from a control value of 7.0. Some regultion seemed to occur between 5 and 20 min of seizure activity. The pH_e underwent an initial, fast acidification during the first minute after seizure induction, followed by a partial recovery, and thereafter the pH_e decreased to reach a stable value around 0.36 units below control within the first 5 min of seizure activity. In hypoxic animals, the pH-changes showed a similar pattern as in the normoxic group, but the decrease of intracellular pH was more pronounced (0.32 units at 5 min seizure activity) and the extracellular acidosis was exaggerated at 20 min with pH_e values 0.5 units below control.

Regarding pH_i, our 5 min seizure values in normoxic animals are similar to those previously reported by other investigators (Howse et al. 1974, Duffy et al. 1975, Chapman et al. 1977) and in accordance with our own previous results (see paper 5). The degree of

intracellular acidosis seen at 20 min of SE, in both normoxic and hypoxic animals, is smaller than can be explained by the lactate accumulation seen at that time (see paper 5, cf. Siesjö 1973, 1978). We propose that extrusion of hydrogen ions occurs from the intracellular space, and that this is possible because there is a persistent and sufficient ATP-source at that time (see paper 5).

Concerning pH_e, the initial acidotic shift, sometimes exceeding 0.4 units, could not be explained by lactic acidosis, since tissue lactate values at 1 min of seizure activity were shown to be only around 6 $\mu mol.g^{-1}$. Previosly, Chapman et al. (1977) demonstrated that bicuculline-induced seizures raised tissue lactate content to 4-6 $\mu mol.g^{-1}$ during the first 30-60 sec. Probably, a spreading depression at seizure initiation is responsible for the initial extracellular acidosis. An acidotic shift in pH_e of $\sim$ 0.4 unit following spreading depression has previously been described by Kraig et al. (1983), and by Mutch and Hansen (1984). A possible explanation for this acidification is that it occurs due to ionic exchanges across cell membranes; i.e. if Na^+ permeability of cell membranes is increased by the seizures, and if Na^+ influx occurs by means of a Na^+/H^+ antiporter, this will result in a fast extracellular acidification. The pH_e values in the following phase of seizure activity (5-20 min) were stable in normoxic rats. However, in the hypoxic animals the increased tissue lactate accumulation (see paper 5) was reflected as an exaggerated acidosis at 20 min of seizure activity. The pH_e changes recorded during seizures were similar to those reported by other investigators (Wang and Sonnenschein 1955, Heuser 1978).

During the recovery phase, pH_i values exceeded controls both at 5 and 20 min, in both normoxic and hypoxic animals. That is, an intracellular alkalosis was at hand. Extracellular pH, on the other hand, remained acidotic during recovery periods up to 65 min, although a pattern of progressive normalization was seen.

The novel findings in this study are as follows: During seizures, there is little or no regulation of the pH_e after the first minutes, and there is a fast, initial, transient and severe acidification of the extracellular space. During recovery, pH_e normalizes at a very slow rate. During recovery, pH_i rapidly normalizes and a postepileptic intracellular alkalosis develops. Previously, Mabe et al. (1983) have reported a similar intracellular alkalosis following ischemia. We suggest that the fast, initial extracellular acidification is due to Na^+/H^+ exchange across cell membranes, and that the intracellular acidosis is caused by lactic acid production. The extrusion of H^+ from the cells might delay and curtail the intracellular acidosis somewhat. The extracellular acidosis seen later during seizures could be influenced by a continuous diffusion of lactic acid from the intracellular space. At seizure arrest, the rapid normalization of pH_i is probably mainly due to a continued extrusion of H^+ (in exchange for Na^+), which together with oxidation of lactate accounts for both the normalization of pH_i and for the subsequent development of intracellular alkalosis. Since lactic acid oxidation is slow, normalization of pH_e is delayed.

CONCLUSIONS

1. Seizures induced by flurothyl cause similar changes in cerebral metabolites, metabolic rate and CBF as bicuculline- and pentylenetetrazol-induced status epilepticus. Thus, the flurothyl model is generally valid as a model for generalized seizures and the histopathological alterations seen after flurothyl seizures are not specific for flurothyl.

2. In the recovery phase after 20 min of seizure activity, cerebral metabolites in the parietal cortex and in the hippocampus normalize at different rates. Cyclic AMP and phosphocreatine regain normal values within 5 min after seizure arrest, whereas glycogen and lactate concentrations display slower normalization.

3. The postictal phase up to 45 min after 20 min of status epilepticus is characterized by a pronounced depression of local CMRgl and CBF in several structures, most conspicuous in the neocortical regions. There is also a local mismatch of CMRgl and CBF.

4. During 20 min of seizure activity, there is an intra- and extracellular acidosis in the parietal cortex. After cessation of seizures, intracellular alkalosis develops, but extracellular acidosis remains even at 45 min of recovery. Since there is a rapid and pronounced reduction of l-CBF in the parietal cortex at seizure arrest, and extracellular pH (pH_e) remains nearly unchanged, pH_e can not be the major factor regulating CBF.

5. Status epilepticus causes brain damage even in ventilated, well-oxygenated rats, where systemic complications like hypotension, hypoglycemia and hyperthermia are avoided.

6. Irreversible damage appears in the substantia nigra pars reticulata (SNPR) and in the globus pallidus (GP) already after seizure periods as short as 30 min. After more prolonged seizure periods, the following structures are affected: the neocortex (45 min), the hippocampal CA_1 and CA_4 regions, the amygdala, the thalamus (60 min) and the cerebellum (90 min). The extent of the damage increases with increased duration of the seizure period. After 2 hrs of seizures, though, damage is mild in all structures except SNPR and GP.

7. Severe local damage with infarction of the tissue is seen only in the SNPR and in the GP, where the lesions arise during ongoing seizures. Thus, there is neither "maturation" of the neuronal damage, nor "recovery" of affected cellular elements after seizure arrest. In the other regions affected, sparse selective neuronal necrosis is seen. We suggest that there are different mechanisms of action underlying these two types of epileptic brain damage: the SNPR/GP lesions are caused by local metabolic failure with excessive lactic acid accumulation, probably due to mitochondrial failure, whereas the selective neuronal necrosis is due to an excitotoxin process.

8. Lesions of the noradrenergic locus coeruleus (LC) system aggravate seizure-induced selective neuronal necrosis in the parietal cortex. We suggest that the extent of neuronal necrosis is modified by activation of the inhibitory LC system during seizures, probably by counteracting neuronal hyperexcitation caused by excessive excitatory transmitter release.

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Irreversible neuronal damage after short periods of status epilepticus

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It is still unknown by what mechanisms sustained or recurrent seizures give rise to irreversible cell damage in the brain. In clinical material, brain damage due to status epilepticus is often localized to regions which are vulnerable to hypoxic/ischemic insults as well, such as the hippocampus, neocortex, and cerebellum (e.g. Corsellis & Meldrum 1976). The possibility remains, therefore, that systemic alterations like hypoxia and hypotension play an important role in the pathogenesis of the epileptic lesions. Although such alterations can be controlled and largely avoided in animal experiments it is still unknown if epileptic activity *per se* gives rise to cell damage. Suggestive evidence is the limbic lesions resulting from local or systemic administration of kainic acid (for literature, see Coyle 1983). However, it is generally held that generalized seizures of less than 90 min duration do not give rise to brain cell damage, and opinions differ as to whether more prolonged seizures do (see Delgado-Escueta et al. 1983). Evidence now exists that neuronal damage due to ischemia "matures" over hours and days (Kirino 1982, Pulsinelli et al. 1982). Thus, some structural alterations observed at the termination of the insult may disappear during recovery while others, notably those reflecting irreversible damage, develop in this period, sometimes after a free interval of days. It is clear, therefore, that assessment of epileptic brain cell damage is only possible if models are developed which allow long-term recovery following seizure periods of defined duration. In this article, we give a preliminary account of results obtained with such a model and report novel information on the neuronal damage incurred.

The experiments were carried out on male Wistar rats (300–400 g) which had free access to pellet food and tap water. After anesthesia with halothane, intubation, placement of tail arterial and venous catheters, and immobilization with suxamethonium, the animals were ventilated on $N_2O:O_2$ (70:30). Body temperature, as measured with a rectal probe, was maintained at 37°C, and arterial P_{O_2} and P_{CO_2} at >100 mmHg and 35–40 mmHg, respectively. Frontoparietal EEG was recorded from needle electrodes inserted subcutaneously and 0.1 mg/kg atropin was given. Seizures were induced by bis-(2,2,2-trifluoroethyl)-ether (Armageddon Chemical Co., Armageddon, PA) a convulsant gas which, when added to the inspired air, elicits generalized seizures within seconds (Krantz 1963, see also Duffy et al. 1975). In order to achieve sustained seizures, 80 µl of the gas were injected into the inlet of the respirator and retained in a rebreathing system, containing a CO_2 absorber. If necessary, one or two additional doses of 20 µl each were given. When seizures were sustained for 15–30 min they could be terminated by withdrawal of the fluorothyl. Status epilepticus of longer duration usually had to be terminated by i.v. injection of thiopenthal (15 $mg \cdot kg^{-1}$). In the recovery period the suxamethonium supply was discontinued and, when their spontaneous respiration was resumed, the animals were extubated. Preliminary experiments showed that adequate physiological parameters could not be upheld in the recovery period. Assuming that this was a late effect of the massive sympathoadrenal discharge during seizures we administered phentolamine i.v. (1.5–2.5 $mg \cdot kg^{-1}$) at seizure onset so as to curtail the ictal hypertension. This pretreatment usually allowed uneventful recovery.

Rats were sacrificed after one week survival by peraortic perfusion with buffered 4% formaldehyde. The brains were dehydrated in graded ethanols, embedded in paraffin, sub-serially sectioned at 8 microns, and double-stained with acid fuchsin and cresylviolet. Direct counting of acidophilic neurons was performed at a magnification of 320.

Flurothyl-induced seizures were accompanied by

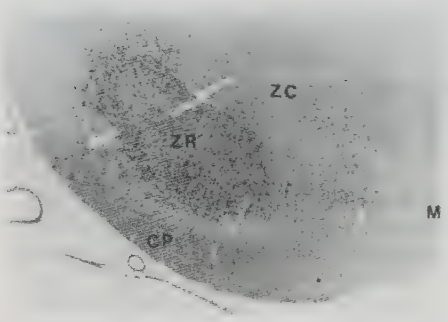

Fig. 1. Midbrain of rat with sixty minutes of status epilepticus and one week survival, showing a hypercellular infarct selectively localized to the substantia nigra, zona reticulata (ZR). The adjacent zona compacta (ZC) of the substantia nigra is preserved. The cerebral peduncle (CP) and midline (M) are indicated. Acid Fuchsin/Cresyl Violet, X50.

EEG changes and systemic alterations similar to those previously recorded in bicucilline-induced status epilepticus (Chapman et al. 1977). Thus, following an initial period of intense spike and wave activity a pattern of burst-suppression was sustained until termination of seizure activity. Administration of phentolamine usually limited the ictal hypertension to 160–170 mmHg but did not cause ictal or postictal hypotension since blood pressure could regularly be maintained above 100 mmHg. Apart from a moderate reduction in P_{CO_2} and a delayed plasma acidosis (pH 7.2–7.3) systemic alterations were slight. With the seizure periods employed (15–90 min) the animals regained consciousness and motility within 1–4 hours. In no animal, seizure activity was observed in the recovery period.

Animals exposed to 15–25 min of seizure activity failed to show evidence of neuronal cell damage. However, such damage was regularly observed after longer periods. As judged from the limited number of experiments performed, a rank order of susceptibility of the rat brain to final cell damage incurred after status epilepticus exists, with the following susceptible structures arranged in order of decreasing selective vulnerability: pallidoreticularis, cerebral cortex (layers 2–3), hippocampal CA4 and CA1 neurons, and thalamic nuclei. The first brain structures affected were the pars reticularis of the substantia nigra and the globus pallidus, showing damage after 30 min of status epilepticus. At all times this damage was extensive, yielding a picture of overt infarction with hypercellularity or cyst formation (Fig. 1). After 60 min seizure activity the cerebral cortex began to show dying neurons, ranging in number from 5 to 70 per whole brain section. Only the rare hippocampal CA1 or CA4 pyramidal neuron showed damage after this seizure duration. After 90 min, substantial numbers of hippocampal CA4, but not CA3 or CA1 neurons were damaged, but the number of "dying" cortical neurons per section did not increase. Acidophilic neurons have been established in other experiments as non-viable by their disappearance from tissue sections after two and four weeks survival.

The present results thus yield novel information of a partly unexpected nature since unequivocal neuronal damage was recorded already after seizure periods of 30–45 min, and since the earliest lesions were observed in the pallido-reticularis, a structure which showed infarction after seizure periods of 30 min, or longer. Several lines of evidence suggest that this result is not peculiar to the seizure model employed. For example, our previous results obtained with bicuculline-induced seizures have shown that glucose utilization in the substantia nigra initially increases to more than 200% of control, and later falls to remarkably low values (Ingvar & Siejsö 1983). In retrospect, this result can be interpreted to indicate metabolic "exhaustion" of reticulate cells in this structure. Similar metabolic results following netylenetetrazol-induced seizures were reported by Howse (1983) who, inaddition, noted extensive histopathologic alterations in the substantia nigra. In itself, this finding is equivocal since other results have demonstrated resolution of such "intra-ictal" alterations during the first 1–2 hours of recovery (Söderfeldt et al. 1983). However, in the light of the present results once can infer that substantia nigra lesions develop following seizures induced by bicuculline, pentylenetetrazol, and fluorothyl.

Since damage to the pallido-reticularis does not seem to be a conspicuous feature of clinical epilepsy the present result may not be a useful paradigm for human disease. However, they demonstrate that unequivocal neuronal damage is incurred after relatively short periods of status epilepticus, seemingly uncomplicated by reduced tissue oxygenation. Furthermore, they focus interest on those functional and/or metabolic characteristics which

render cells in the pallido-reticularis markedly vulnerable to enhanced neuronal activity.

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Status Epilepticus in Well-Oxygenated Rats Causes Neuronal Necrosis

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Neuronal necrosis in the brain resulting from status epilepticus of 15 to 120 minutes duration in ventilated and well-oxygenated rats was assessed. Seizures were induced by inhalation of the convulsant gas flurothyl, and terminated by withdrawal of flurothyl and a single injection of thiopental. The animals were allowed to recover for one week, and neuronal damage was assessed by cell counts following subserial sectioning of the brain and microscopical examination of the sections. Infarction of the pars reticulata of the substantia nigra occurred in 5 of the 6 animals with seizure duration of 30 minutes, and in all animals with longer seizure durations. There also was a common affectation of the central parts of the globus pallidus. The pars compacta of the substantia nigra was never affected. After 45 to 120 minutes of seizures, moderate neuronal necrosis was observed in the neocortex (layers 3 and 4), and after 60 to 120 minutes was seen in amygdaloid and thalamic nuclei, as well as in CA4 and CA1 hippocampal pyramidal cells. Notably, CA3 neurons were not damaged nor were dentate granule cells affected. After 120 minutes of seizures, damage regularly affected the neocortex and the ventral–posterior nuclei of the thalamus. A conspicuous feature was the localization of neuronal necrosis at sites close to the ventricles.

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Patients with repeated or sustained epileptic seizures, or both, may incur brain damage that is often localized to the neurons of the hippocampus, neocortex, thalamus, and amygdala [11]. It remains unknown if this damage results from the seizure discharge per se, from complicating tissue hypoxia, or from a combination of both [29, 44]. Some studies suggest that repeated or sustained seizures need not cause neuronal necrosis. For example, experiments in rats, in which three daily electroshocks produced up to a total of 140 seizures, failed to reveal damage to hippocampal pyramidal cells [33]. Furthermore, other studies in which seizures were sustained up to 5 hours in other mammals yielded equally negative results [5, 14, 16, 42]. On the other hand, results obtained in baboons indicate that, if seizures are sustained more than 90 minutes, damage will be incurred even if the animals are prevented from developing arterial hypoxia, hypotension, or hyperthermia [30, 31]. A recent study with amygdaloid stimulation following the establishment of a kindled focus demonstrated brain damage when seizures were prolonged for 4 hours or longer [28].

Controversy also surrounds the problem of localizing the epileptic neuronal necrosis. A body of evidence derived from experiments employing kainic acid, presumed to mimic temporal lobe epilepsy, suggests that, although many brain areas may suffer damage, the chief target is the CA3 pyramidal cells in the hippocampus ([3, 4]; see especially [12]).

A drawback of most previous studies is that at best brief recovery was allowed before brain damage was assessed. Results obtained in ischemia, however, demonstrate that the final cell damage "matures" over hours and days, neuronal necrosis often being preceded by a free interval [22, 25, 40]. Another potential pitfall is that structural alterations (e.g., those resembling ischemic cell change), observed in tissue fixed by perfusion during or shortly after the insult, can be fully reversible [47].

In order to study the final neuronal damage after status epilepticus, we devised a recovery model for sustained seizures, employing the convulsant gas flurothyl, and allowed one week of recovery before perfusion fixation of the brain. The present study, which gives the results of experiments with seizure durations of up to 120 minutes, shows that seizures sustained for such periods produce unequivocal and predictably localized brain damage. A preliminary account of some of the experiments with short duration has been published [35].

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Materials and Methods

Animal Groups

The experimental groups were subjected to 0 (controls, n = 6), 15 (n = 6), 30 (n = 6), 45 (n = 3), 60 (n = 6), 90 (n = 6), and 120 (n = 6) minutes of status epilepticus followed by recovery for one week.

Chemicals

Flurothyl (hexafluorodiethyl ether) was supplied by Armageddon, Inc (Armageddon, OH). Phentolamine was diluted to 0.8 mg/ml before use (Regitin; Ciba Geigy, Basel, Switzerland). Atropine and suxamethonium were diluted in Krebs solution to 0.05 mg/ml and 5 mg/ml, respectively, before use (ACO Läkemedel, Stockholm, Sweden). All other chemicals used were of reagent grade.

Animal Procedures

The animals (330 to 430 gm) were male Wistar rats, SPF strain (Møllegaard's Avslaboratorium, Copenhagen, Denmark). Free access to pellet food (Astra-Ewos, Södertälje, Sweden) and tap water was allowed until the time of experiment. The rats were anesthetized in a jar with a mixture of 3% halothane in 30% oxygen/70% nitrous oxide. When unresponsive, they were intubated and ventilated on a Starling-type ventilator (Braun, Melsungen, West Germany). Paralysis was maintained with intermittent injections of suxamethonium (3 mg per kilogram of body weight), and the halothane was decreased to 0.7%. The tail artery was cannulated for recurrent monitoring of blood gases (Eschweiler & Co, Kiel, West Germany), pH (Radiometer, Copenhagen, Denmark), and blood glucose (Ames Co, Elkhart, IN), and constant monitoring of blood pressure (Siemens Elema, Stockholm) and regulation of blood pressure by exsanguination (described in next section). A catheter was inserted in one tail vein for infusion of drugs, glucose, and blood (to be described). Scalp electrodes were used for single-channel electroencephalographic (EEG) monitoring (Siemens Elema, Stockholm). The rectal core temperature was regulated to approximately 37°C. Upon completion of the operative procedure, atropine was given (0.05 to 0.1 mg/kg) to minimize airway secretion, and halothane was discontinued. At least thirty minutes was allowed for physiological stabilization and elimination of the halothane before the seizures were instigated.

Induction and Maintenance of Seizures

The animals were connected to a rebreathing ventilator system with a serially coupled carbon dioxide absorber containing a gas mixture of 60% nitrous oxide/40% oxygen. Oxygen supply was regulated manually using the volume of the expansion balloon in the system as the index, and was checked by monitoring of blood gases. To minimize the ictal increase of blood pressure at the start of the seizure period, 3 to 5 ml of blood was withdrawn. Phentolamine was then infused until the blood pressure decreased to 100 to 120 mm Hg (2 to 5 min). The flurothyl was then added by injection directly into the rebreathing system (80 μl). Blood pressure was then maintained at 110 mm Hg or higher for the duration of the seizure period by reinfusion of the shed and donor blood (up to 8 ml in the longest experiments). In order to maintain ictal activity, additional boluses of flurothyl (20 to 40 μl) were injected as required to sustain a burst-suppression EEG pattern for the duration of the seizure period.

During and after the seizure period, blood gases were monitored and blood oxygen tension was maintained at greater than 90 mm Hg, and carbon dioxide tension was kept at between 30 and 40 mm Hg. The blood glucose level was also checked using reagent strips and a reflectance meter (Ames Co, Slough, England), and it was maintained above 5 μmol/ml by infusion of a 25% glucose solution into the tail vein as required.

Recovery from the Seizure Period

Seizure activity stopped spontaneously when the flurothyl supply was discontinued in the 15-minute group and in 3 of 6 animals in the 30-minute group. In most of the animals subjected to more prolonged seizures, it was necessary to arrest the seizures with a short-acting barbiturate (intravenous thiopental, 15 $mg \cdot kg^{-1}$). When spontaneous breathing movements returned, the nitrous oxide was discontinued and the animals were extubated. Blood gases were obtained in order to check the adequacy of the respiration. During the following week the animals were checked with daily weight measurements, and their general status was recorded (e.g., feeding, drinking, grooming).

Histological Procedures

One week after status epilepticus, the animals were reanesthetized with halothane, intubated, and, after isotonic saline flushing, were perfusion fixed with 4% formaldehyde solution in a 0.1 M phosphate buffer (400 ml). The brain was left in situ for 24 hours before removal. After coronal sectioning into 2.8-mm-thick slices, the brains were dehydrated in graded ethanols, cleared in xylol, and embedded in paraffin. The entire brain was subserially sectioned at 8 μm. Sections were double-stained with 0.1% cresyl violet followed by 1% acid fuchsin fortified with glacial acetic acid [1, 2]. The resulting coronal brain sections thus obtained were examined and direct visual counting of acidophilic, cytoclastic neurons was performed for the cerebral cortex and hippocampus. The distribution of acidophilic neurons was noted throughout the entire brain. The spinal cord was not examined.

Results

The Table summarizes the physiological variables during and after the seizure period. The status of the animals during recovery was related to the duration of the seizures. The longer the seizures the slower the recovery.

During the two hours following extubation, the animals remained quiet, with little spontaneous movement and reduced or absent reaction to stimulation. Recovery followed gradually, without evident hyperexcitability or overt seizures.

Within 2 to 3 days the animals appeared normal (except for one noise-sensitive rat in the 120-minute group). All rats were able to eat and drink within 24 hours.

Physiological Variables for the Animals in the Two-hour Seizure Group[a]

Sample Time	Temp (°C)	MABP (mm Hg)	PaO_2[b] (mm Hg)	pH[b]
Before seizures	36.5 ± 0.2	127 ± 4	100 ± 4	7.37 ± 0.01
115 minutes of seizures	37.2 ± 0.1	124 ± 6	120 ± 1	7.29 ± 0.01
90–120 minutes of recovery	37.1 ± 0.1	133 ± 6	116 ± 9	7.34 ± 0.02

[a]The values given represent samples taken before the start of the seizure period, at the end of the seizure period, and 90 to 120 minutes after the ictal activity had ended. Values are given as mean ± SE.
[b]PaO_2 and pH were measured arterially.

MABP = mean arterial blood pressure; PaO_2 = arterial oxygen tension.

Fig 1. The areas with the major affectation of the different types of neuronal necrosis in rat brain subjected to 120 minutes of status epilepticus and allowed one week of recovery. A *indicates selective neuronal necrosis with the neuroanatomical pattern;* B *indicates pannecrosis (all cellular elements); and* C *shows areas with the periventricular pattern of selective neuronal necrosis.*

All animals lost weight in proportion to the length of the seizure period, averaging 21% in the 120-minute group and 9% in the 15-minute group.

Acidophilic neurons were seen in numerous supratentorial and infratentorial structures. Such neurons have been found to undergo cytolysis and removal from tissue sections with survival longer than one week after an insult, and also have electron microscopical characteristics indicating necrosis [1].

Two distinct patterns of brain damage were seen. The first conformed to certain nuclei and cortical laminae of the brain, and the second pattern showed a relationship to the cerebral ventricles and aqueduct. These will be referred to as the neuroanatomical and periventricular patterns, respectively, and are shown in Figure 1.

Neuroanatomical Pattern

CEREBRAL CORTEX. Status epilepticus of 120 minutes was required to regularly produce cortical damage; 2 out of 3 animals showed sparse acidophilic neuronal necrosis after 45 minutes, but 2 out of 6 in the 90-minute group had no such necrotic neurons. In the latter animals, the number of necrotic neurons varied between 0 and 92 in the sections in which cell counts were done. In the 120-minute animals, the corresponding numbers for damaged neurons were 47 to 338, suggesting progressive damage with longer seizures. The damage never progressed beyond selective neuronal necrosis to complete tissue infarction, even in the most severely affected cortical areas.

Cortical neuronal necrosis chiefly affected lamina 4 (Fig 2), although occasional acidophilic neurons also appeared in other laminae. Next affected were the pyramidal cells of layers 3 and 2, and lastly, layers 5 and 6 neurons. Necrosis of layer 6 neurons was distinctly unusual, except for locations near the lateral ventricles.

Damage tended to be worst over the superolateral convexity of the hemisphere and extended from the frontal cortex to the occipital cortex. The entorhinal cortex and cingulate cortex were less severely involved than was the neocortex of the convexity.

HIPPOCAMPUS. The CA1 and CA4 regions in some animals showed necrotic neurons after 60 minutes of status epilepticus. Acidophilic CA4 cells were distributed throughout the entire hilus of the dentate gyrus, but some neurons were always preserved, even in the most severely affected animals. Often the CA1 region was spared (Fig 3).

In the CA1 region, neuronal necrosis occurred with roughly equal frequency along the length of the cell band, from the subiculum to the border of CA3. The

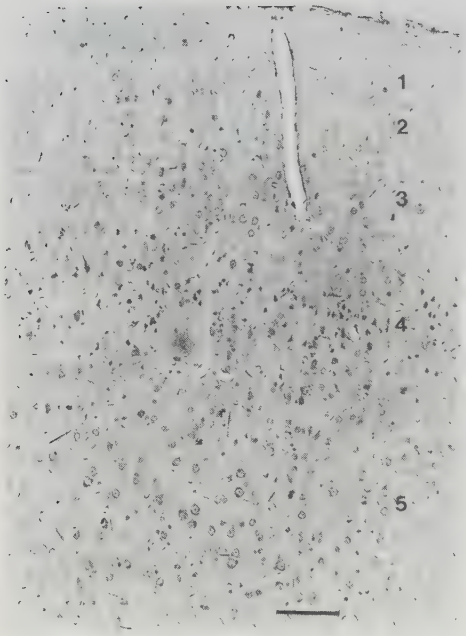
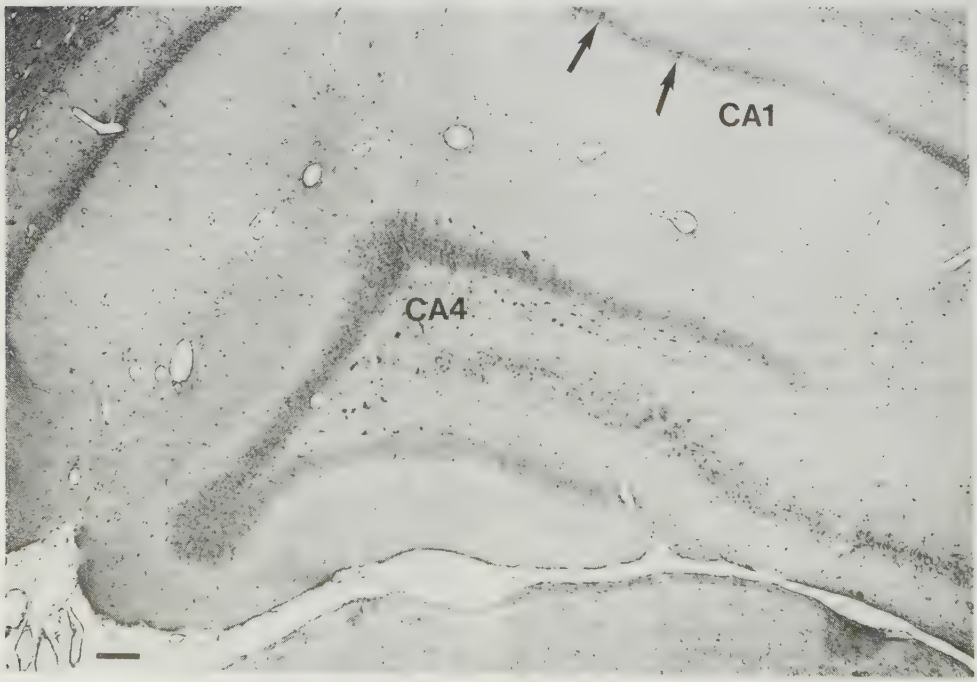


Fig 2. Neocortex, one week after 120 minutes of status epilepticus. Necrotic neurons stain vividly with acid fuchsin and are dark and punctate. Necrosis predominates in lamina 4, with only a few necrotic neurons in other laminae. (Acid fuchsin–cresyl violet; bar = 200 μm.)

interneurons of the stratum radiatum and stratum oriens were occasionally affected. The CA3 pyramidal neurons and granule cells of the dentate gyrus were uniformly spared.

BASAL GANGLIA. The different nuclei varied widely in their damage, the globus pallidus being more susceptible than the caudoputamen. Neuronal necrosis in the globus pallidus appeared after 30 minutes of status epilepticus in 3 of 6 animals, and in all animals after 120 minutes of status epilepticus. Unlike the solely neuronal lesions seen in the cerebral cortex, the le-

Fig 3. Hippocampus, one week after 120 minutes of status epilepticus. Neurons undergoing acidophilic neuronal necrosis predominate in CA4, although a few individual necrotic neurons are present in CA1 (arrows). *(Acid fuchsin–cresyl violet; bar = 150 μm.)*



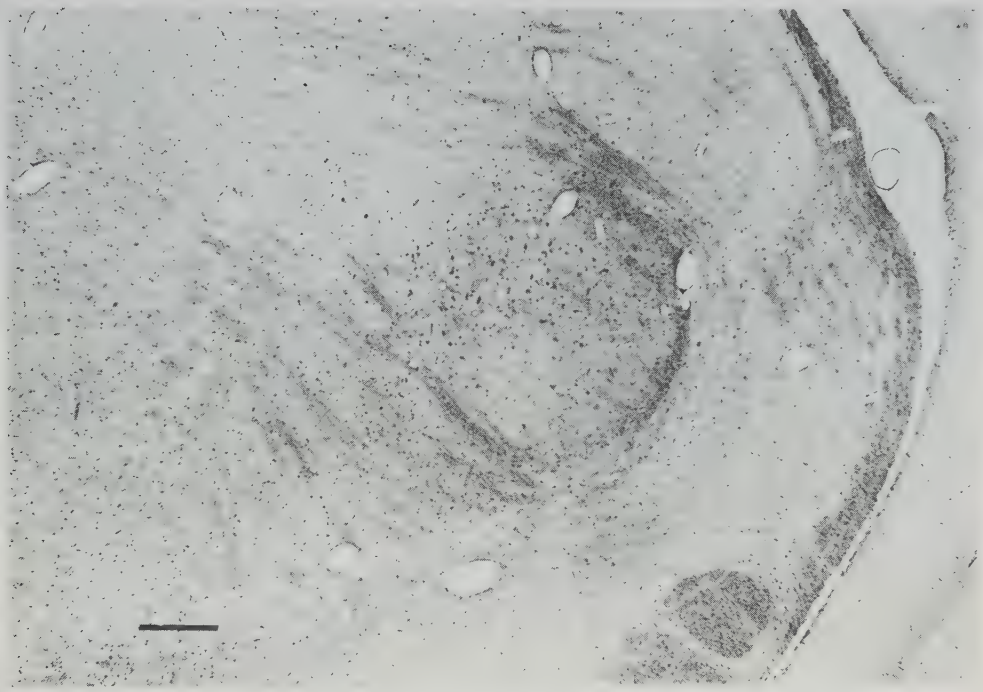


Fig 4. Posterior thalamus, one week after recovery from 120 minutes of seizures. This photomicrograph demonstrates nuclear affectation of the ventral posterior cell group lying within the superior thalamic radiation. (Acid fuchsin–cresyl violet; bar = 400 μm.)

sions of the globus pallidus were characterized by spongiosis of the neuropil. Within this spongy area, normal neurons were uncommon whereas the vascular elements seemed normal. The lesions tended to be symmetrical and often affected the lateral part of the nucleus, involving most of the pallidum in the severely affected animals. Infarction occurred only in the center of the most severe lesions, being surrounded by spongiosis of the neuropil.

The caudate nucleus was involved after 120 minutes of status epilepticus, but in only 2 of 6 animals. Damage comprised randomly distributed selective neuronal necrosis of small, medium, and large neurons.

THALAMUS. Crisply demarcated acidophilic neuronal necrosis was seen in several thalamic nuclei. Most commonly affected were the ventral posteromedial and ventral posterolateral [38] relay nuclei (Fig 4). The posterior nuclear group and the medial geniculate body were less regularly affected, and the reticular nucleus was always spared.

MIDBRAIN. Large bilateral symmetrical infarcts were seen in the pars reticulata of the substantia nigra (SNPR) (Fig 5) in 3 of 6 animals after 30 minutes of status epilepticus, and in all but 1 animal with longer seizure periods. Selective neuronal necrosis was not seen in this area. All animals with 15 minutes and one with 30 minutes of status epilepticus displayed no damage in the pars reticulata. Two animals in the 30-minute group had small (20% of the nucleus) lesions that were in the center of the SNPR. EEG recording during the seizure period provided no indication of which animals would develop an infarct. Thiopental was needed to end seizure activity in all but one animal that had developed a large SNPR infarct. The size of the infarct bore no apparent relation to duration in animals with more than 30 minutes of status epilepticus.

The SNPR infarcts were oval-shaped, and the medial and lateral portions of the nucleus often showed some preserved neurons. The lesion never extended into the adjacent pars compacta but sometimes extended perivascularly into the crus cerebri (Fig 5). Infarcts were characterized by a hypercellular infiltrate of

Fig 5. Mesencephalon, demonstrating the characteristic hypercellular infarction of the pars reticulata of the substantia nigra (SNPR) one week after 120 minutes of status epilepticus. Note the sparing of the neurons in the borders of the SNPR, and the perivascular spread of the infarct into the cerebral peduncle (arrows). *(Acid fuchsin–cresyl violet; bar = 200 μm.)*

macrophages surrounding a core with a paucity of cells. A vacuolated neuropil was present for a few microns only at the perimeter of the lesion, resulting in a sharp demarcation. The outlines of acidophilic neurons were visible in the degenerating and somewhat acidophilic neuropil. No neuroglial or vascular elements were clearly discernible.

CEREBELLUM. The lateral lobes of the neocerebellum showed occasional acidophilic Purkinje neurons (Fig 6), first appearing after 90 minutes of status epilepticus. Many of these were only slightly shrunken, but demonstrated a pronounced affinity for acid dyes.

Fig 6. Cerebellar cortex, taken from the lateral lobe one week after 120 minutes of seizures. Purkinje cells undergoing acidophilic neuronal necrosis are seen. (Acid fuchsin–cresyl violet; bar = 200 μm.)

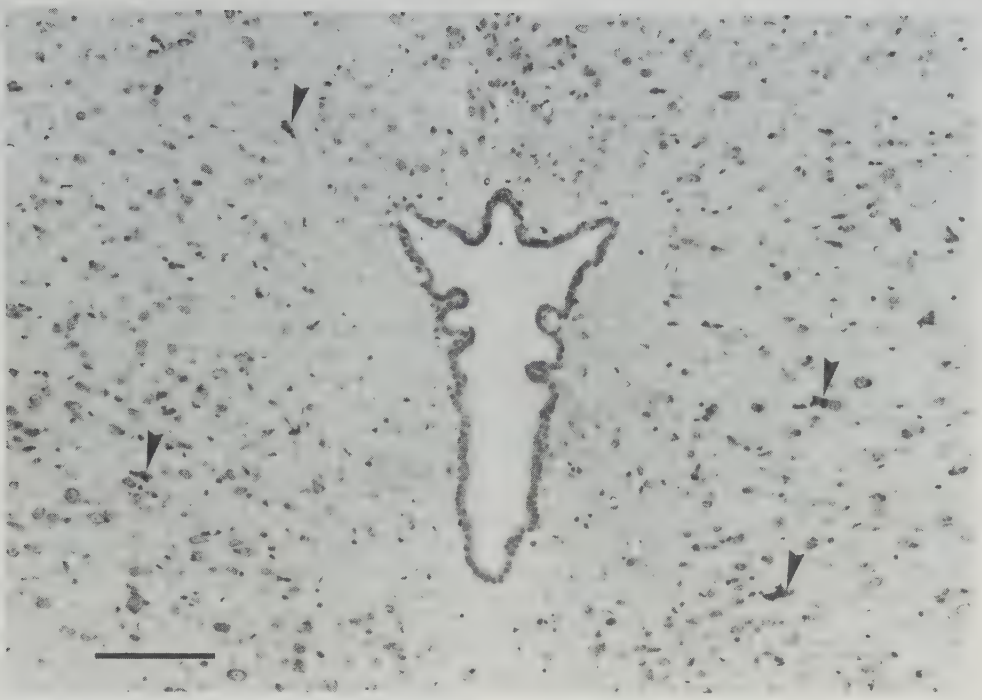


Fig 7. The periventricular pattern of damage in the periaqueductal gray matter, from the levels of the mesencephalon, one week after 120 minutes of seizures. Some of the numerous neuronophagic figures are indicated with arrowheads. (Acid fuchsin/cresyl violet; bar = 100 μm.)

Periventricular Pattern

The periventricular pattern developed clearly only in animals subjected to seizures longer than 90 minutes, although a few necrotic neurons with this localization pattern were seen in the 30-minute group.

Near the lateral ventricles, several acidophilic necrotic neurons were present in lamina 6 of the neocortex, but only in periventricular locations where there was scant intervening white matter of the centrum semiovale (see Fig 1). The neuronal necrosis tapered rapidly as the white matter increased in width superiorly.

The lateral and basal amygdaloid nuclei were affected [38], but there a subanatomical distribution was seen. The portions of the nuclei nearer the ventricles showed greater numbers of acidophilic neurons.

Close to the third ventricle, both superior and inferior to the interthalamic adhesion, periventricular acidophilic neuronal necrosis was seen in the thalamus, involving a wide array of nuclei that surrounded the ventricle. Moderate acidophilic neuronal necrosis surrounded the periaqueductal gray matter, being more common superiorly toward the third ventricle where the aqueduct is larger (Fig 7). Acidophilic Purkinje cells near the outlet foramina of the fourth ventricle were limited to the side of the cerebellar folium facing the foramen of Luschka.

Discussion

The Model

It has been suggested that flurothyl opens sodium channels in the cell membrane [48] with no specific population of neurons identified as being specifically sensitive. The gas has previously been used to induce short-lasting or repeated seizures in acute experiments [13, 26, 41], but this is the first small animal model for status epilepticus allowing prolonged recovery. Since flurothyl is administered as a gas, the model is different from other pharmacologically induced types of status epilepticus. Flurothyl is quickly exhaled after discontinuation of supply. As a result, seizures of less than 30 minutes' duration stop spontaneously, and with status epilepticus of longer duration, the seizures can be arrested permanently by a single dose of thiopental (15.

mg/kg). This abrupt cessation contrasts with the use of bicuculline to induce prolonged seizures after which one must use large doses of diazepam to terminate seizure activity, making recovery longer than a few hours impossible [47]. All animals studied in the present experiments had adequate ventilation and systemic circulation, and they were all able to drink and eat within 24 hours postictally.

Necrosis of the SNPR

Flurothyl seizures in rats [13, 15] induce metabolic changes similar to those obtained with convulsants such as pentylenetetrazole and bicuculline [6, 13, 21]. One can only speculate that the distribution of neuronal damage is similar, since data are lacking for such an extended recovery period as with flurothyl [47]. Evidence exists, however, that pentylenetetrazole and bicuculline also cause SNPR lesions. The histological results presented by Howse [18] imply that necrosis developed by the end of a 90-minute pentylenetetrazole-induced status epilepticus, and our still unpublished data demonstrate that similar SNPR lesions can be induced by bicuculline, pentylenetetrazole, or flurothyl. Also, these agents cause a similar metabolic activation pattern, as judged by carbon 14–labeled 2-deoxyglucose autoradiography [18, 20; Ingvar and Nevander, unpublished results, 1985]. In the beginning of the seizure period the SNPR displays a very marked metabolic activation, followed by a depression after the first 30 minutes of seizures. The time course for this dynamic change is the same in all three models.

Metabolic activation of the SNPR is common to other seizure models, that is, when systemically administered kainic acid is used [10] and when focal seizures are induced by focal cortical penicillin injections in rats [7–9] or monkeys [17, 24]. However, the metabolic depression seen late in seizures and the necrosis of the SNPR have not been reported in these focal models. We conclude that the damage in the SNPR is not "flurothyl-specific" but rather is a lesion common to status epilepticus of several causes, including γ-aminobutyric acid (GABA)–receptor blocking (bicuculline), antagonism of GABA-mediated postsynaptic inhibition (pentylenetetrazole), or opening of neuronal sodium channels (flurothyl) [49]. Iadorala and Gale [19] postulated that the substantia nigra functions as a central gate for the generalization of any kind of seizure activity ("a critical midbrain site for GABA-mediated anticonvulsant activity"). The data presented here support the concept that the SNPR may be critical for the seizure spread. The time in these experiments when the flurothyl-induced seizures became self-sustained seems to coincide with the time when the infarction develops in the SNPR. Thus all animals but one that had a large infarction in the SNPR after one week of recovery required thiopental to arrest the seizure activity, but none of the other animals needed thiopental to arrest the seizure activity.

Neuropathological studies of human material obtained after death following status epilepticus [32, 36] have not revealed lesions of the SNPR comparable to those occurring in well-ventilated rats subjected to status epilepticus. Hence, this lesion, but not the metabolic activation during seizures, may be unique to rodents, possibly requiring adequate supplies of oxygen and glucose for its development. Nevertheless, the present results suggest that increased attention be directed to the pars reticulata of the substantia nigra, and the globus pallidus in seizure states.

Anatomical Considerations in Neuronal Loss

The periventricular pattern of neuronal necrosis is reminiscent of the distribution pattern of hypoglycemic damage. In this condition, the distribution has tentatively been assumed to be mediated by a fluid-borne toxin [2]. The present results indicate that small amounts of such a toxin might be released into the ventricular system due to the intense neuronal activity during status epilepticus giving rise to periventricular neuronal necrosis.

The neuroanatomical pattern of damage in these animals is clearly different from that which follows, for example, the systemic injection of kainic acid [4, 10], a substance that causes focal status epilepticus of limbic lobe structures. Kainic acid–induced damage is localized to the limbic system, especially CA3 in the hippocampal formation [4, 27, 34]. This excitotoxin [37] has a more regionally specific effect, as the most vulnerable areas seem to correlate with the local distribution of a specific, high-affinity, saturable kainate receptor [12, 45]. With flurothyl, neuronal damage in the hippocampus is most prominent in the CA4 and CA1 zones, leaving the CA3 intact. In short-lasting ischemia in gerbils and rats, several authors have shown the CA3 to be less vulnerable than the CA4 and CA1 [22, 25, 40, 46], in a pattern similar to the flurothyl model. We find it unlikely, however, that the damage in status epilepticus is the result of ischemia. Measurements of cerebral energy metabolites, blood flow, and metabolic rate during seizures show that at least the cortex and the hippocampus have an adequate blood flow and an energy state close to normal [15, 20, 43; Ingvar and Nevander, unpublished results, 1985].

Status epilepticus inflicted much less neuronal damage on the hippocampus than we expected. The present animals had an adequate blood pressure both during seizures and in the recovery period. As the vascular autoregulation during seizures is lost [39], the possibility of hypotensive periods occurring in status epilepticus could have contributed to the hippocampal

lesions reported in the clinical and experimental literature [11].

One must ask whether excitatory pathways recruit cell damage at postsynaptic sites [3, 9, 27]. Previous deoxyglucose studies provide examples of metabolic activation that could possibly be responsible for both pre- and post-synaptic damage [20; Ingvar and Nevander, unpublished data, 1985]. Thus, the damage incurred in the thalamus was most extensive in the ventral posterior nuclei. Those nuclei project to cortical layers 3 and 4 (the specific thalamocortical tract [23]), which also displayed neuronal loss. However, our results do not answer the question of whether this transsynaptic damage is orthodromic or antidromic in nature [9]. The damage in the hippocampus may represent an affectation of the end station of the activation of the entorhinal cortex (via the perforant path [50]).

Not even a very marked metabolic activation can by itself explain the damage incurred, as several hypermetabolic structures ([20]; Ingvar and Nevander, in preparation) are intact after 120 minutes of seizure activity. It seems established, however, that the activation of a structure is a prerequisite for any histological damage due to seizures. Presumably both the type of agonist released by presynaptic terminals and the characteristics of the postsynaptic receptor represent important determinants of whether neuronal loss develops.

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THE DEVELOPMENT OF HYPERMETABOLIC INFARCTION IN THE SUBSTANTIA NIGRA IN STATUS EPILEPTICUS: 2. MORPHOLOGY

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ABSTRACT

The topographic distribution and the temporal evolution of infarction of the pars reticulata of the substantia nigra and of the globus pallidus secondary to flurothyl induced status epilepticus were examined by light and electron microscopy. The lesions were found to develop in the centers of both nuclei, with perivascular spread seen. Selective neuronal necrosis was not observed, and mild damage showed only microvacuolation of the neuropil, sparing neurons.

Electron microscopy of the evolving infarct revealed sequential pathologic changes involving first the axons, then dendrites, neurons, glia, and outer pericytes, but universally sparing endothelial cells. Swollen mitochondria were followed by local rupture of cell membranes, leading to cavitation of the neuropil. Edema, measured densitometrically, was absent.

The findings indicate that infarction of the pars reticulata of the substantia nigra in the rat in status epilepticus is a spreading process beginning in axonal elements of the synaptic neuropil, and axons of passage. A pronounced hypermetabolic local enhancement of glycolysis leads to a focal lactic acidosis. We speculate that central accumulation of lactic acid occurs, with drainage via the perivascular space and the white matter. The globus pallidus, being richer in white matter and hence drainage of interstitial fluid, shows less damage after a similar metabolic activation. Preservation of endothelial cells and often inner pericytes distinguishes this form of infarction from the changes of autolysis and middle cerebral artery occlusion. The term hypermetabolic infarction is suggested for this lesion.

Although the pathophysiology of cerebral infarction has been intensively studied in the past years, its pathogenesis remains unclear. Infarction of central nervous tissue is a cavitating process seen in cerebral ischemia, either temporary or permanent. In that situation, carbohydrate loading, with excessive accumulation of lactic acid in the tissue has been shown to promote cerebral infarction (28,45,46,52,61,62,70,81).

In addition to lactic acid accumulation, edema has been considered important in the pathogenesis of cerebral infarction (52). The converse of this has also been proposed. Cerebral necrosis has been held to be necessary in the pathogenesis of subsequent ischemic brain edema (59,60). The specific role of ischemic edema in the pathogenesis of cerebral infarction is thus not firmly established.

In rats subjected to 60 or more min of status epilepticus, selective neuronal necrosis is regularly seen in the cerebral cortex, hippocampus, and other brain regions. Infarction is also seen, in the substantia nigra pars reticulata (SNPR), produced by only 30-45 min of status epilepticus (53,54). These lesions allow observations of the process of infarction to be made in a wider context than in ischemia alone. Marked hypermetabolism has been documented in the SNPR, preceeding the development of infarction due to status epilepticus (36, Nevander and Ingvar, in preparation). After 30 min of status epilepticus, the metabolic rate decreases to nearly zero. However, no parallel decrease in the cerebral blood flow is seen during this time (36, Nevander and Ingvar, in preparation).

It is known that a deterioration of the cellular energy state occurs concomitant with a pronounced focal lactic acidosis in the substantia nigra of rats with flurothyl-induced status epilepticus for 60 min (37). Lactic acid accumulation to 25 μmol/g has been shown (37). In the present article we describe the morphologic counterpart of these metabolic findings, and their temporal sequence.

To study the role of edema in infarct development, regional water content was measured with gravimetric methods in the substantia nigra, globus pallidus, and cerebral cortex during and shortly after seizures. Edema was found not to contribute to the infarction.

A spreading process is demonstrated, beginning in the axonal elements, later involving dendrites, and still later involving other cellular elements. In this form of infarction, termed hypermetabolic, endothelial cells are spared.

Materials and Methods

A previous article describes more completely the model used (54). Briefly, 18 fed male Wistar rats (330 g - 430 g) were anesthetized in 3% halothane in nitrous oxide/oxygen (70/30%), intubated, ventilated on a Starling type ventilator, and paralysed with suxamethonium. The halothane was decreased to 0.7%, and the tail artery and vein were cannulated for monitoring of blood pressure, blood gases, blood glucose, and for infusion of drugs, glucose and blood. Blood pressure was regulated by exsanguination and reinfusion of blood. The EEG was monitored by scalp electrodes. The rat was maintained at 37°C, given atropine, and the halothane was discontinued. Thirty min were allowed before seizures were instigated by the introduction of flurothyl into a rebreathing ventilator system (54). To minimize the ictal increase of blood pressure at the start of the seizure period, 3 to 5 ml of blood were withdrawn and phentolamine was infused. The flurothyl was now added by injection directly into the rebreathing system (80 µl). The blood pressure was then maintained at 100-120 mm Hg for the duration of the seizure period by reinfusion of the shed blood and donor blood (up to 7 ml in the longest experiments). In order to maintain ictal activity, additional boluses of flurothyl (20-40 µl) were injected as required to sustain a burst-suppression EEG pattern

for the duration of the seizure period.

Blood gases and blood glucose levels were monitored and were maintained within the ranges described in a previous paper (54) during and after the seizure period. Seizures terminated spontaneously when the flurothyl supply was discontinued in most animals, but a short acting barbiturate (pentobarbital iv 15 mg/kg) was occasionally required to stop seizure activity. When spontaneous breathing movements returned the N_2O was discontinued, blood gases were checked, and the animals were extubated.

Histological procedures

Eighteen rats were perfused after no epilepsy (control), 20, 35, 45, and 60 min of seizure activity, and after 1, 3, 6, 24 hrs and one week recovery following 60 min status epilepticus. One to three animals were studied after each survival interval. Ten additional animals were used to assess regional water content, as described below.

Perfusion fixation was carried out on the tracheotomised or re-intubated rat, during mechanical ventilation with 1% halothane in a 2:1 mixture of $N_2O:O_2$. Heparin (90 I.U.) was given into the left ventricle, and a cannula dripping saline was introduced into the ascending aorta. The cannula was firmly sutured in place, the right auricle was opened, and normal buffered saline at 37°C was flushed through the cerebral circulation for 30 sec. Following this, 3% phosphate-buffered glutaraldehyde at pH 7.35 was perfused through the cerebral circulation for 30 min. The brains were removed from the skull after several hours, and stored in fixative until processing.

The fixed brains were grossly examined and cut into roughly 1 mm slices. Samples of the substantia nigra and globus pallidus were manually dissected from the slices for both light and electron microscopy, from both sides of the brain. Bilateral samples were taken from each animal to

give five thick sections from each region, and one or two of these were selected for thin sectioning and electron microscopy. The remaining portions of the lenticular nuclei and midbrain were examined by light microscopy.

For light microscopy, tissue was dehydrated in graded ethanols and embedded in a soft methacrylate polymer (EFL-67, Serva Feinbiochemica, Heidelberg, F.R.G.), sectioned at 2 μm, and stained with either Acid fuchsin/Cresyl violet, or toluidine blue only.

For electron microscopy, sections were dehydrated and embedded in Epon 812. Sections were stained with uranyl acetate and lead citrate, and viewed on a JEOL JEM 100C electron microscope.

Measurement of Tissue Water Content

The animals comprising this portion of the study are depicted in Table 1. Water content was assessed during the status epilepticus, and one hour thereafter. After decapitation, the brain was immediately removed from the skull. Tissue samples were taken from the fresh cut brain in a moisture chamber containing an atmosphere with a relative humidity of 96%. Samples were excised from the surfaces of cut brain slices, and were taken from the geographic center of the globus pallidus and substantia nigra, pars reticulata.

The microgravimetric technique of Tengvar et al (77,78) was used to measure tissue edema. Linear density gradients of polyvinyl pyrrolidine coated silica particles (Percoll, Pharmacia Fine Chemicals, Uppsala, Sweden) in 0.125 M sucrose/0.15 M NaCl were prepared in 250 ml graduate cylinders. The flotation points of colored glass spherules of known specific gravity were used to establish the linearity of the gradient, and to create a calibration curve. The excised tissue samples were introduced into the gradient. When the buoyant density level was reached, the

Table 1

Gravimetric edema measurements during status epilepticus

Experiment	substantia nigra, pars reticulata	globus pallidus	cerebral cortex
Control	1.043	1.043	1.043
Control	1.040	1.045	1.041
Control	1.043	1.042	1.043
Control	1.045	1.045	1.041
20′ Epilepsy	1.041	1.040	1.042
20′ + 25′	1.038	1.038	1.042
60′	1.042	1.042	1.044
60′	1.046	1.041	1.040
60′ + 60′	1.044	1.043	1.044
60′ + 60′	1.042	1.041	1.042

position in the cylinder was noted and the specific gravity was determined from the calibration curve for the gradient at that time.

Results

Substantia Nigra - Light Microscopy

As described previously (54), infarction of the substantia nigra pars reticulata was regularly seen one week after 60 min of status epilepticus (Fig 1). The infarct involved the geographic center of the nucleus, sparing a peripheral rim of neurons.

The first alteration visible by light microscopy was pallor of the neuropil in the substantia nigra pars reticulata (Fig 1). This was seen when the tissue was stained with acidic or basic dyes (eg. toluidine blue). At several days recovery or longer, there was an increased affinity for acid dyes, which involved both neurons and intervening neuropil.

Transformation of neurons into dark cells was seen. Microvacuolation of neuronal cytoplasm was common in these dark neurons, and in otherwise normal neurons. These changes appeared already after 20 min of status epilepticus, and persisted throughout the period of epilepsy.

Between one day and one week, macrophages infiltrated the peripheral rim of the infarct (Fig 1B). Collapse of the necrotic central core proceeded after one week, leaving only a gliotic scar corresponding to the location of the pars reticulata of the substantia nigra (50).

Substantia Nigra - Electron Microscopy

Control material from the pars reticulata of the substantia nigra, and of the globus pallidus revealed tissue consisting chiefly of synaptic neuropil, with a paucity of neurons (25,31,65,69). Axonal boutons terminaux were arranged around dendritic processes in a rosette-like

arrangement, completely covering the dendrites. Neurons with abundant mitochondria and cytoplasmic organelles were seen.

Pars Reticulata of the Substantia Nigra

1. Synaptic Neuropil

The first alterations were visible in the axons of the synaptic neuropil, already after 20 min status epilepticus. Axonal boutons terminaux forming axo-dendritic or axo-somatic synapses, as well as axons en passant, either myelinated or unmyelinated, showed swollen mitochondria with disordered and fractured cristae. Dendrites were spared, and the mitochondria in dendrites trans-synaptic to pathologic axons were relatively normal (Fig 2A).

After 35 min, dendrites as well as axons became involved, and by 45 min, there was damage to nearly all dendrites (Fig 2B). After one hour of seizures, axons and dendrites were indiscriminately affected, demonstrating swollen and ruptured mitochondria with fractured cristae. These mitochondria often contained flocculent densities.

Cell membrane breaks began at 35 min (Fig 2B), and were ubiquitous after 45 min to one hour (Fig 3). Confluence of spaces resulting from membrane breaks caused enlarging cavities in the tissue. The lesion thus evolved into a pan-necrosis through the first hour of status epilepticus by progressively augmenting cavitation beginning in the neuropil.

Axonal shrinkage and resorption was seen to occur inside intact myelin sheaths (Fig 4, 5B). Subsequent tissue breakdown was accompanied by infiltration of macrophages over the following week after the insult.

2. Neurons

Dark neuron transformation occurred in some neurons of the pars reticulata of the substantia nigra already after 20 min (Fig 6), but was

more common after 45 min. These neurons had electron dense nuclei and cytoplasm, and showed ubiquitous and severe mitochondrial swelling (Fig 6, inset). However, nuclear and cytoplasmic membrane integrity was still preserved. Many lethally injured neurons never underwent dark cell transformation (Fig 3). Neuronal cell membrane breaks and cytorrhexis were seen after 45 min in both dark and neurons of normal electron density, with herniation of cytoplasmic contents into the extracellular space. Mitochondrial flocculent densities were present in such cells (Fig 3).

In the recovery period, neurons were either in the advanced stages of cell breakdown, or were completely normal. Neurons did not appear selectively vulnerable; even after one day, surviving neurons were seen, often surrounded by cavitating neuropil (Fig 4).

3. Glia

Astrocytes and oligodendrocytes either showed signs of necrosis, or survived with little reactive change. By one hour of epilepsy, there were mitochondrial flocculent densities and cell membrane rupture, both indices of cell necrosis. Surviving cells showed little reactive change.

Astrocytic cell bodies, like neuronal perikarya, did not seem selectively vulnerable. Surviving glial cells were often surrounded by cellular debris (Fig 4).

4. Vascular changes

Although vascular changes were seen, the cellular elements comprising blood vessels were not equally affected. Pericytes, situated outside the endothelial basal lamina, were often necrotic, whereas endothelial cells, situated inside the basal lamina, showed only reactive changes. Among several pericytes surrounding the same blood vessel, some showed complete cytolysis and others were normal (Fig 5A). Pericyte necrosis was marked by

mitochondrial flocculent densities (Fig 5B).

In many vessels, several layers of pericytes were present in concentric layers, each completely enclosed by a basal lamina. Most commonly, outer ones were necrotic and inner ones were preserved in such situations (Fig 5C).

Endothelial cells demonstrated reactive changes, but no necrotic endothelial cells were found in any animal, in spite of surrounding cavitation of the neuropil (Fig 5D). The reactive changes consisted of endothelial pits and vesicles, and prominent endothelial microvilli.

Globus Pallidus - Light Microscopy

Light microscopy of the globus pallidus showed a lesion beginning in the gray matter intervening between the large white matter fascicles of the pallidum. There was pallor of the neuropil during the status epilepticus, followed by microvacuolation which developed after several hours to several days survival.

In the globus pallidus, the infarct also began near the center of the nucleus in mildly affected animals, and in more severely affected animals spread to involve progressively more of the nucleus. Most of the pallidum was involved in animals showing a well-developed lesion.

The appearance of the lesion between one day and one week was determined by its severity. Mild damage was apparent as microvacuolation of the neuropil, with a conspicuous sparing of neuronal cell bodies (Fig 7A). More severe damage was marked by a macrophage infiltration selectively into necrotic gray matter, sparing the preserved intervening white matter (Fig 7B). Only in the most severely affected animals did a pan-necrosis develop, which engulfed the large fascicles of the pallidum and the intervening neuropil indiscriminately (not shown).

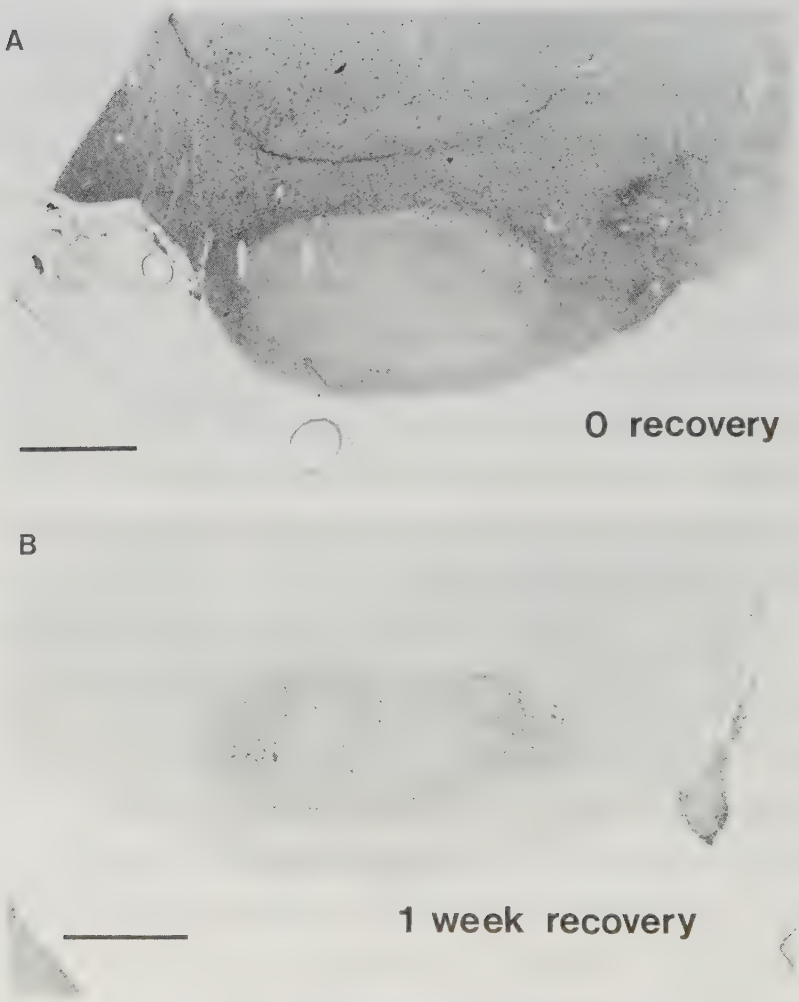


Fig 1. At the end of 45 min of status epilepticus (A), the substantia nigra shows an oval, crisply demarcated region of tinctorial pallor. Gravimetric measurements of tissue water revealed no edema at this time (see Table 1). (B) After one week recovery, an infarct is seen as a peripheral macrophage infiltrate surrounding a core of coagulative necrosis. The perimeter of the nucleus is spared, and does not show a zone of selective neuronal necrosis. (A) Toluidine blue. (B) Acid fuchsin/Cresyl violet. Bars = 1 mm.

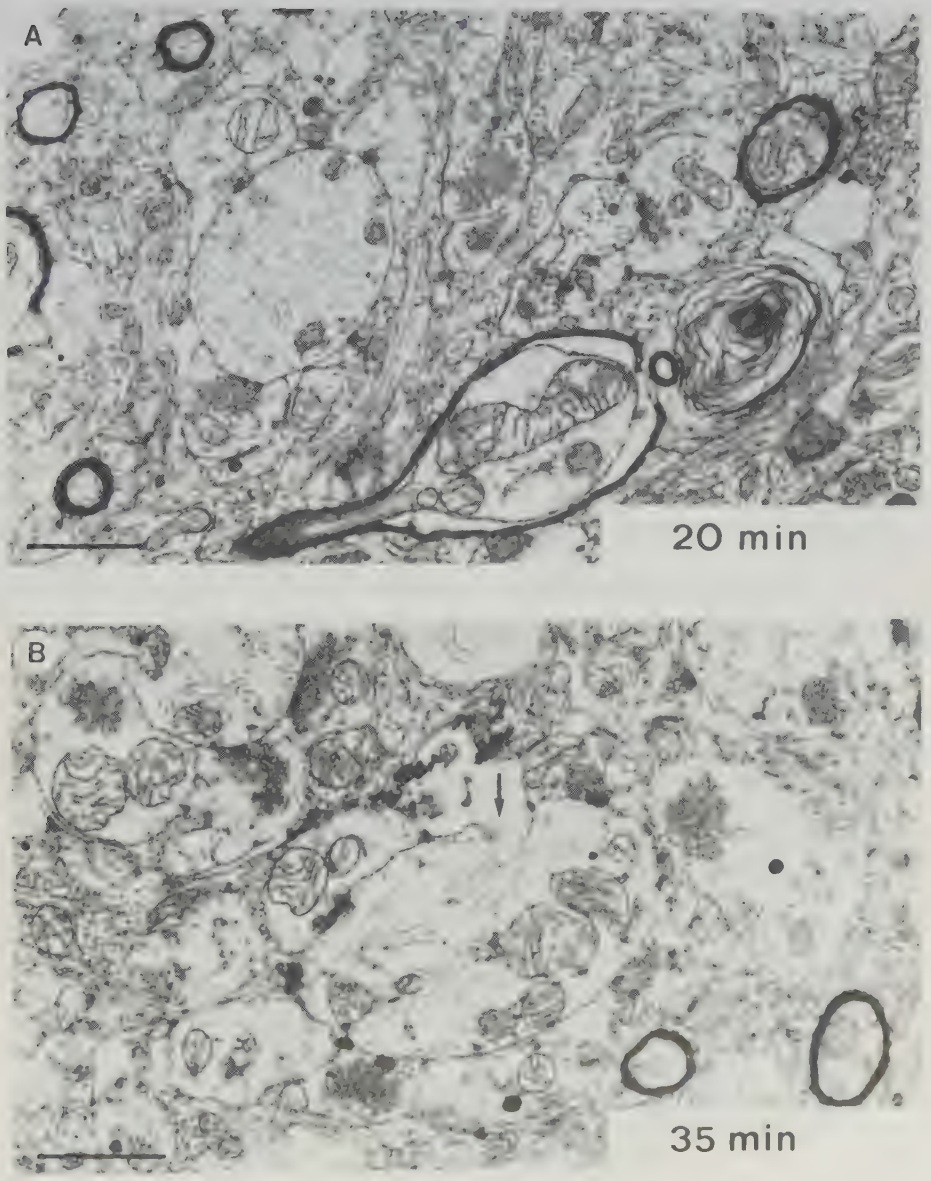


Fig 2. Synaptic neuropil after 20 min and 35 min status epilepticus. The lesion begins in the axons as swelling and mitochondrial swelling. Dendrites are normal, but axons of passage as well as terminal axons are affected. By 35 min, synaptic membranes have begun to rupture (arrow). Substantia nigra, pars reticulata. Bar = 1 μm.

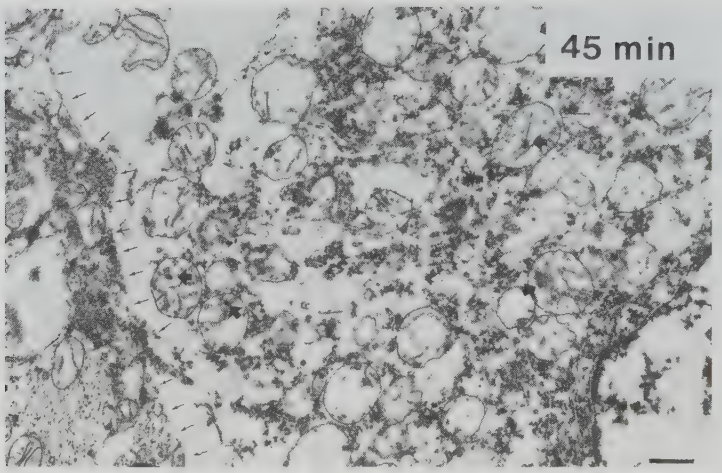


Fig 3. Signs of cell necrosis are seen already after 45 min of status epilepticus. This neuron, which did not undergo dark cell transformation (cf Fig 6), shows mitochondrial flocculent densities (broad arrows) and a ruptured cell membrane (thin arrows). Bar = 1 µm.

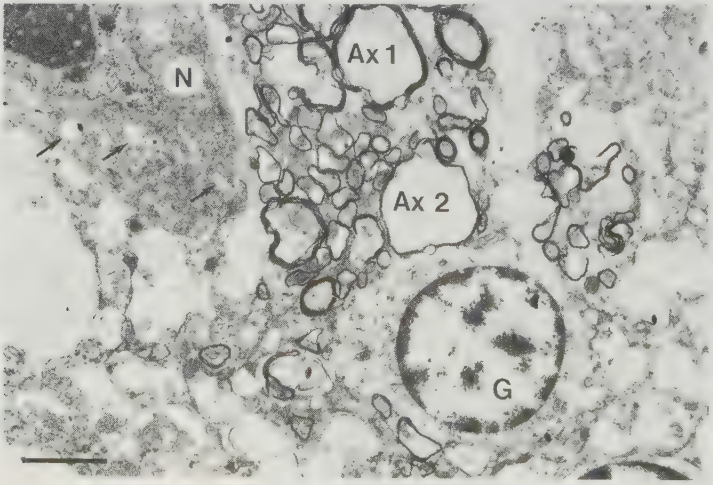
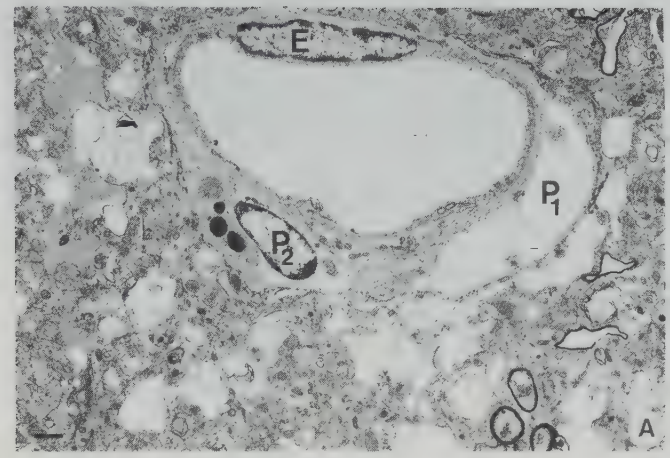
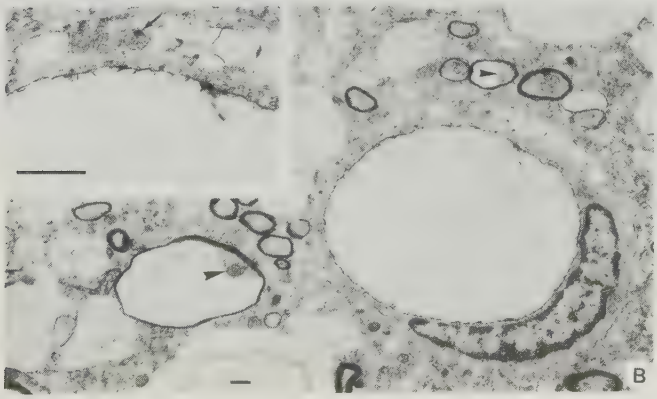


Fig 4. Relative preservation of neuronal (N) and glial (G) cell bodies is apparent. Only a few microvacuoles are seen in the neuronal cytoplasm, due to mitochondrial swelling (arrows). The surviving cells are surrounded by cavities resulting from membrane rupture in the neuropil. Axons are undergoing severe shrinkage (Ax 1), or are totally resorbed (Ax 2) within their myelin sheaths. 60′ epilepsy + 60′ survival. Bar = 5 µm.

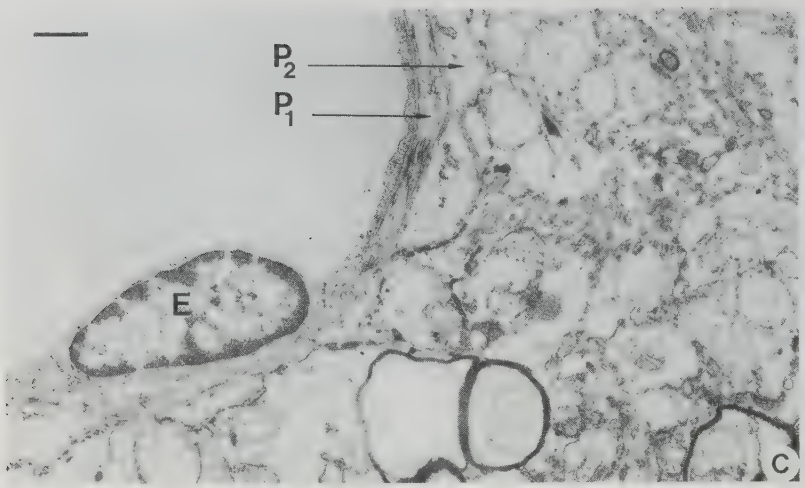
Fig 5. Vascular changes.



A. Endothelial cells (E) show no necrosis, but pericytes (P), enclosed within a basal lamina external to the endothelium, are sometimes necrotic. One pericyte (P_1) shows complete cytolysis, whereas a surviving lipid-laden pericyte (P_2) is also present in the same vessel. The surrounding neuropil shows pan-necrosis.



B. Cavitation of moderate degree surrounds a surviving endothelial cell and a necrotic pericyte, higher magnification of which (inset) reveals a flocculent density (arrow) in a mitochondrion. Resorbing axons are seen within intact myelin sheaths (arrowheads). Fibrin, staining darkly, is adherent to the vessel lumen.



C. Selective necrosis of the outer pericyte (P1) with preservation of the inner pericyte (P2). The endothelial cell (E) is intact, and surrounded by necrotic neuropil.

D. Endothelial cell (E) survival in spite of severe cavitation of the neuropil. Only a few membranes are left in the neuropil surrounding the capillary. A necrotic oligodendrocyte is seen at the lower left of the picture.

Bars = 1 μm.

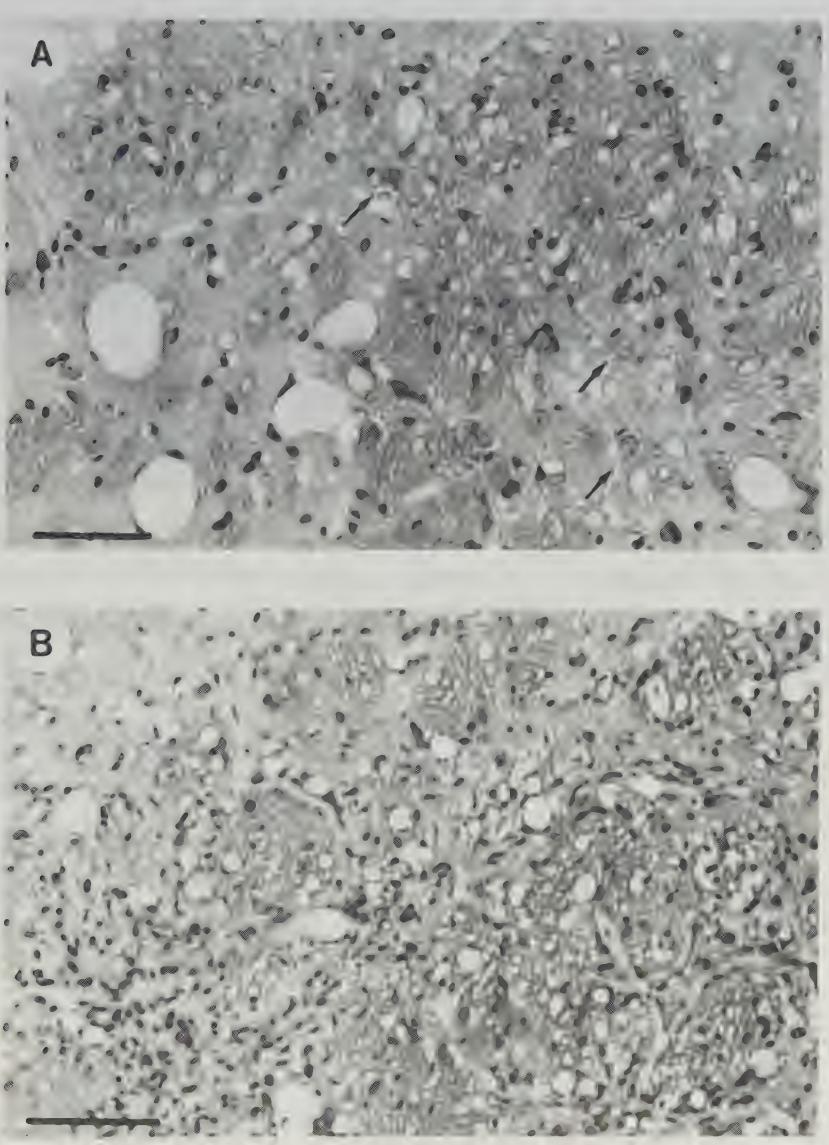


Fig 7. Globus pallidus, showing a minimal lesion (A), and a more severe lesion. The minimal lesion shows only vacuolation of the neuropil, and a conspicuous sparing of neurons (arrows). The more severe lesion (B) shows dense infiltration of gray matter by macrophages, outlining the fascicles of white matter. Acid fuchsin/Cresyl violet. Bars = 100 μm.

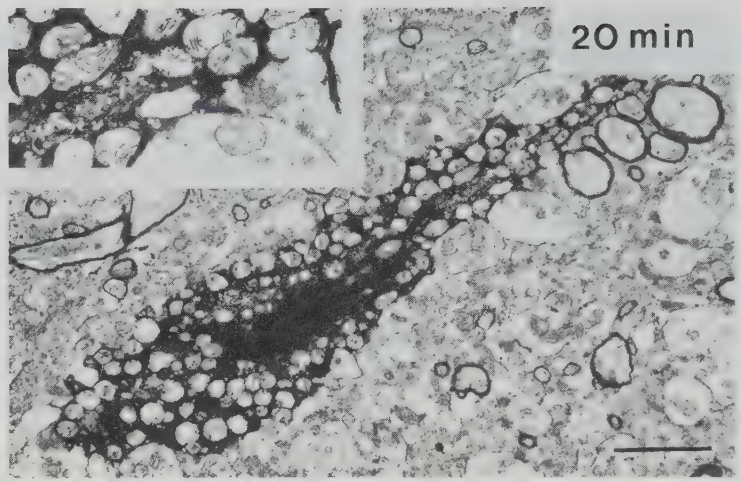


Fig 6. Dark neuron transformation is seen already after 20 min status epilepticus. Virtually the entire cellular population of mitochondria is affected by severe swelling of mitochondria and disruption of cristae (inset). Bar = 8 μm.

Fig 8. Globus pallidus after 45 min status epilepticus. Axons, including myelinated ones, show severe swelling of mitochondria, and are undergoing resorption within intact myelin sheaths (arrowheads). However, the large fascicles of white matter are spared (inset). As in the substantia nigra, pericytes (P) are affected, but there is sparing of endothelium (E). Bar = 2 μm.

Globus Pallidus - Electron Microscopy

Electron microscopy showed changes in the synaptic neuropil of the globus pallidus similar to those in the substantia nigra. Axons, both myelinated and unmyelinated, terminal and en passant, initially showed only swollen mitochondria, but by 45 min began shrinking within their intact myelin sheaths (Fig 8). In contrast, the large myelinated fascicles of white matter in the globus pallidus were unaffected (Fig 8, inset).

Prominent microvacuolation was seen in pallidal neurons during the period of status epilepticus. Electron microscopy showed these to be swollen mitochondria, as in the pars reticulata of the substantia nigra. Subsequent cavitation, however, was mild to minimal, dependent on sampling. As in the substantia nigra, pericytes were affected, but endothelial cells spared (Fig 8).

Measurement of Brain Density Changes

Results of measurement of tissue edema by flotation of freshly excised samples of cerebral cortex, globus pallidus, and substantia nigra in density gradient solutions are presented in Table 1. Neither during the epilepsy, nor after one hour recovery, was tissue edema detectable.

Discussion

The results will be discussed within the general framework of selective vulnerability in the brain, followed by a specific consideration of pathophysiologic events leading to the present infarcts. Lastly, we will discuss the results in a wider context, comparing the morphologic changes with those seen after permanent, complete ischemia and autolysis, and with changes seen after administration of excitotoxins.

Selective Vulnerability

The globus pallidus and the pars reticulata of the substantia nigra (pallido-reticularis) are related embryologically (51,72) and cytoarchitecturally (25,69). They have a similar selective vulnerability to several diseases processes, including carbon monoxide poisoning (11) and Hallervorden-Spatz disease (24). The globus pallidus and pars reticulata of the substantia nigra are thus often considered as a single structure, this connotation being carried in the abbreviation "pallido-reticularis".

In experimental well ventilated status epilepticus the pallido-reticularis is susceptible to damage (35,53,54). Both the substantia nigra and the globus pallidus undergo metabolic activation shortly after the onset of seizures, followed by metabolic silence (36). These metabolic changes are more pronounced in the substantia nigra, and tissue damage is also more severe in that structure. Only the pars reticulata of the substantia nigra develops into an infarct after periods of epilepsy as short as 30 min. Longer periods are required to produce infarcts in the globus pallidus (54).

Initiation of Infarction

Infarction in the classic sense, consisting of cavitation of CNS tissue and macrophage infiltration, was seen in the substantia nigra, pars reticulata in the present study. This structure consists chiefly of synaptic neuropil, neuronal cell bodies being relatively sparse (31,42,47,65,69).

A sequential process was observed in the present study, beginning in axons, both myelinated and unmyelinated axons en passant, as well as in presynaptic terminal axonal boutons. Axonal mitochondria were swollen, and contained fractured and disorganized cristae. Dendrites were spared at

this time, after 20 min of status epilepticus.

Pathologic changes then spread sequentially to involve all remaining cellular elements except endothelium. Dendrites became involved, and membranes began to rupture between pre- and postsynaptic elements of the neuropil, leading to cavitation. Neuronal and glial cell bodies were relatively spared, and were often surrounded by confluent cavities. The resorption of axons within intact myelin sheaths further indicates this is a primary intra-axonal process, rather than being secondary to external events in the surrounding neuropil.

Proposed Role of Lactic Acid Accumulation and the Spread of Infarction

Previously, hypermetabolism was shown to accompany the present infarcts (36,37). Hypermetabolism occurs already at 20 min epilepsy, when pathologic changes are still minimal. The early and primary lesion thus appears to be a hypermetabolic disorder beginning structurally in the axons. Other tissue elements are secondarily recruited. Since myelinated axons of passage function solely in electrical conduction and lack receptors for chemical transmitters, electrical rather than receptor mediated chemical events initiate the lesion. We propose that excessive ion pumping across axonal membranes leads to local energy failure, seen as ballooned mitochondria. Glycolysis is enhanced out of proportion to oxidative metabolism, resulting in an accumulation of lactic acid (37). Lactic acid itself is probably responsible for the spread of the pathological process from the axons to other cellular elements within the SNPR.

A possible explanation of the differential selective vulnerability between the pars reticulata of the substantia nigra and the globus pallidus lies in a differential lactic acid accumulation in the two structures. Cerebral white matter serves as a low impedance conduit for

drainage of interstitial fluid from the brain (12,19,22,48,67,73), and white matter is abundant in the globus pallidus. Drainage of lactic acid-bearing interstitial fluid via the perivascular spaces (16,74) may explain the perivascular damage sometimes seen in the crus cerebri adjacent to the substantia nigra (54). The central location of the lesions in both nuclei is possibly due to exacerbation of lactic acid accumulation centrally.

The focal lactic acidosis might also account for the alterations in stainable protein constituents of the tissue, appearing histologically as tinctorial pallor (Fig 1A). Such protein alterations probably represent denaturation, since they coincide temporally with the appearance of necrosis ultrastructurally: Mitochondrial flocculent densities, which represent denatured mitochondrial proteins (19), were seen already after 45 min epilepsy.

At the time of initiation of infarction, corresponding lactic acid levels in the tissue are 15 μmol/g, whereas plasma lactic acid levels are 4 μmol/ml (17). A gradient thus exists between tissue and blood lactic acid levels, and presumably, between cerebral extracellular and plasma H^+ activities. In the vascular cells, there was a corresponding gradient of necrosis, the outer cells being more affected. Pericytes are vascular cells situated outside the endothelium, in one or several concentric layers, completely enclosed within their own basal lamina (47,57). When several layers of pericytes were present, in the characteristic onion-skin arrangement, there was relative preservation of the inner pericytes nearer the lumen. These locations nearer to the intact microcirculation are located "downstream" the lactic acid level gradient from the neuropil, the site of lactic acid production. Endothelial cells themselves, being directly exposed to the moving blood stream, are still further "downhill" the lactic acid concentration gradient.

Lactic acid is implicated in the genesis of brain infarction in other settings as well. Cerebral infarction in a non-vascular distribution is seen in disorders of mitochondrial respiratory chain enzymes resulting in lactate accmulation (49,63).

The histologic character of the lesions reported here resemble those of Wernicke's encephalopathy (thiamine deficiency). Both show microcavitating necrosis (64,80) of the neuropil, giving a vacuolated, spongy appearance to the tissue by light microscopy (7,32,33,80). Neuronal cell bodies are relatively spared (7,21,23), and pericytes are also preserved (7). The central portions of vulnerable brain regions are most severely affected (33,82), and involvement of the pallido-reticularis has been shown in Wernicke's disease in monkeys (64). Lactic acid accumulation in thiamine deficiency has long been known (58), but a focal lactic acidosis in the selectively vulnerable regions has only recently been demonstrated (34). The two lesions thus appear morphologically and chemically similar, both featuring focal neuropil destruction related to lactic acid accumulation in the affected brain regions.

Role of Edema

Edema has been considered important in the pathogenesis of cerebral infarction (52). The present results show that edema, measured as a change in brain density, was not present during the development of the infarct, and thus net water uptake plays no role in the pathogenesis of the lesions observed. The histologic appearance of tinctorial pallor and clear vacuoles in the tissue initially suggested edema to be present (3). However, the gravimetric measurements of the present study revealed no increase in tissue water content. Altered tinctorial affinity of normally stainable tissue constituents for histological stains seems a more likely explanation for the pallor. Since proteins and nucleic acids constitute

the major stainable tissue constituents, early alterations in these components of the tissue may explain the tinctorial pallor.

The histologic appearance of fluid filled spaces (microvacuoles) likewise cannot be attributed to edema. These microcavities appear due to destruction of membranes causing confluence of intra and extracellular spaces, without a net increase in tissue water content. The tissue vacuolation in Wernicke's encephalopathy has also been interpreted as edema (64,66), although the present findings suggest these spaces may be due to cavitation, rather than edema.

Dark Neurons and Cell Death

It has been asserted that dark neurons in tissue sections are always artefacts due to tissue edema and/or poor perfusion fixation (9,15). In the present study, the densitometric measurements of brain tissue demonstrated no changes indicative of edema. Furthermore, difficulty in perfusion fixation is theoretically unlikely, as cerebral blood flow to the substantia nigra is increased, rather than decreased during the development of this lesion (36). Lastly, red blood cells were uniformly absent from all tissue sections.

The dark neurons seen in the present study, occurring focally in an area of selective vulnerability, are thus not explicable on the basis of edema and poor fixation. Rather, dark neurons appear to represent acute injury in several energy failure states (1,2,4,8,39,44,75,76). Microvacuolation of dark neurons, and neurons of normal electron density was seen in the present study, and was due to mitochondrial swelling, confirming previous findings (12,13). However, mitochondrial swelling was less frequent in the mildly damaged globus pallidus.

Death of neurons and glial cells occurred during the insult. They either were killed immediately, or survived. There was thus no "recovery" or

"maturation" of the damage (38) in this lesion. Neurons seemed equally or only slightly more vulnerable than astrocytes or oligodendrocytes. Often, neuronal or glial cell bodies were seen surviving in the midst of necrotic debris and cavitation.

Mitochondrial flocculent densities are a hallmark of irreversible cellular injury (4,27,40,43,79). They were useful in differentiating simple cell swelling from cytolysis and cell death especially in pericytes, since these cells are enclosed within the subendothelial basal lamina and hence cannot rupture into the extracellular space when cell membrane breaks and cytorrhexis develop. Mitochondrial flocculent densities represent denatured proteins of the mitochondrial matrix or inner membrane (19).

Hypermetabolic Infarction vs Autolysis

A comparison of the present findings with previous descriptions of infarction (14,26) and autolysis (43) reveals differences. Whereas autolysis (43) or infarction due to permanent cerebral ischemia (26) are characterised by endothelial necrosis, this was not seen here, nor in the Levine preparation (14). The process of hypermetabolic infarction can thus be considered a necrobiotic process, requiring an intact and functioning microcirculation to develop its morphologic features, distinct from those of autolysis.

Comparison with Excitotoxic Pathology

The CNS tissue lesions seen after administration of compounds known as excitotoxins selectively affect dendrites, sparing axons. This is due to the binding of such compounds to dendritic receptors (20,55), where they open ionic channels and kill neurons. Excitotoxic pathology thus consists of axon sparing, dendritic lesions (55,56). Damaged mitochondria are seen

in the dendrites, and probably represent local energy failure in the dendrite due to excessive membrane depolarization and ion pumping. Epilepsy (29), electrical hyperstimulation (56,71), hypoglycemia (5), and ischemia (41) are excitotoxic processes beginning in neuronal dendrites. Selective neuronal necrosis is seen, with sparing of glia.

The present study showed the converse of the axon-sparing dendritic lesions characteristic of excitotoxins. Damaged mitochondria appeared first in the axons, including myelinated axons, sparing dendrites. This suggests electrical events to be fundamentally important in the pathogenesis of this lesion, as electrical transmission of action potentials is the cardinal function of myelinated axons. The exploded intra-axonal mitochondrias likely represent local energy failure due to excessive membrane depolarization. An excitotoxic process thus does not appear to initiate the infarct. Selective neuronal necrosis was not seen in this lesion, neither in its mildest form, nor at the periphery of the areas of frank infarction.

Cavitation, the essential and destructive feature of infarction, began in the neuropil (axons, dendrites and glial processes) of the pars reticulata of the substantia nigra. Dendrites are of dopamine containing neurons located in the adjacent pars compacta, (6,42,65), activating neurons of the pars reticulata (68). Striatal (30,31) afferents form the major axonal component. Axons form rosette-like arrangements, the axonal boutons surrounding and completely ensheathing the dendrites (31,69). The present lesion began in the axons of this synaptic neuropil. However, damage did not remain restricted to axons but rapidly spread to dendrites and neural and non-neuronal cell bodies, recruiting the entire tissue into a pan-necrosis.

Excitotoxic and hypermetabolic pathogenetic sequences thus appear to be mutually exclusive, the former causing only selective neuronal necrosis, but the latter capable of leading to infarction.

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MECHANISMS OF EPILEPTIC BRAIN DAMAGE: EVIDENCE FOR A PROTECTIVE ROLE OF THE NORADRENERGIC LOCUS COERULEUS SYSTEM IN THE RAT

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Key words: Noradrenaline, locus loeruleus, status epilepticus, neuronal damage, neocortex

Summary:
This study explores the possibility that the noradrenergic locus coeruleus system influences epileptic brain damage. Bilateral 6-hydroxydopamine lesions of the locus coeruleus projection to the forebrain were found to aggravate neuronal necrosis in the neocortex following 60 min of flurothyl-induced status epilepticus. We propose that the activation of the inhibitory locus coeruleus system during status epilepticus counteracts a deleterious neuronal hyperexcitation, probably induced by excessive release of excitatory amino acids, thereby limiting neuronal necrosis.

Experimental evidence has accumulated during recent years indicating that the noradrenergic locus coeruleus (LC) system, which innervates most regions of the CNS [12], has a seizure suppressant action in the brain. Selective lesions of the ascending projections from the LC facilitate the development of kindling [1,6] and potentiate the seizures induced by pentylenetetrazol and electroconvulsive shock [13]. Stimulation of the LC in rats has been reported to suppress pentylenetetrazol-induced epileptiform cortical EEG activity [11]. Degeneration of the LC terminals around the focus precedes the onset of seizure activity in cobalt-induced

epilepsy [19]. Furthermore, the reinnervation of the perifocal area with noradrenergic fibers parallels the disappearence of the epileptic syndrome in the cobalt model [19].

It has not been investigated if the LC system also influences the degree of brain damage following an epileptic insult. This seems highly warranted since it has been suggested [5] that selective neuronal necrosis after prolonged seizures is due to excessive release of excitatory amino acids in the vulnerable areas. The LC system, a principal inhibitory system, is activated during seizures [4,9,10] which, hypothetically, could counteract the deleterious neuronal hyperexcitation, thereby limiting the neuronal necrosis. Such a mechanism was previously suggested to underly the protective action of the LC system found in ischemia induced brain damage [3]. The objective of the present study was to clarify if lesions of the LC system influence seizure induced neuronal necrosis.

The experiments were carried out on male Wistar rats (S.P.F. strain; Møllegaards Avlslaboratorium, Copenhagen Denmark) weighing 300-400g. Bilateral lesions of the LC projection to the forebrain were accomplished in eight animals by 6-hydroxydopamine injections (8 µg in 4 µl saline) into the dorsal catecholamine bundle in the caudal mesencephalon (for details, see [7]). In six rats, which served as a sham group, the cannula was lowered to the same coordinates but no injection was made. After 13-22 days all animals were subjected to 60 min of flurothyl induced status epilepticus according to the method described in detail elsewhere [14]. In brief, the rats were anesthetized with halothane in 70% nitrous oxide and 30% oxygen, paralyzed with succinylcholine and ventilated to maintain arterial pCO_2 between 35 and 40 mmHg and pO_2 around 100 mmHg. Body

temperature was kept close to 37°C. The halothane supply was discontinued and at least 30 min passed before the induction of seizures. To avoid the increase in blood pressure upon the start of the seizure activity, 2.5-7 ml blood were withdrawn and 0.3-0.6 mg phentolamine was injected i.v. Status epilepticus was induced by injection of 80 µl flurothyl (hexafluorodiethyl ether, Armageddon Inc, Armageddon, Ohio) directely into the rebreathing system. During the period of seizures the blood withdrawn was reinfused and the blood pressure was kept above 100 mmHg. After 60 min of status epilepticus the rats were disconnected from the rebreathing system and the seizure activity was terminated by a single injection of thiopental (15 mg/kg i.v.). When spontaneous respiration had returned, the animals were extubated and the adequacy of respiration was checked by blood gas analysis.

Following one week of recovery, the animals were reanesthetized and about 5-15 mg of frontal cortex tissue removed on each side. The noradrenaline (NA) content was later determined using a radioenzymatic method on an acidic extract [17]. The brains were then perfusion fixed with 4% formaldehyde, embedded in paraffin, sectioned coronally at 8 µm and stained with cresyl violet and acid fuchsin (for details, see [2]).

In order to quantify the epileptic brain damage, sectioning intervals were adapted to obtain standard levels of the caudoputamen, neocortex and hippocampus. The counting of the necrotic neurons were carried out in a blind manner at a magnification of 320 x. With the stain employed, necrotic neurons are visible as bright red masses of cytoplasm, often containing deep red staining nuclei, and sometimes surrounded by macrophages. The parietal cortex was analyzed at the

level of the subfornical organ, from the cingulate cortex over the lateral hemispheres to the rhinal fissure. The acidophilic neurons in the caudoputamen were counted at the level of the septal nuclei at their widest point. The hippocampus was quantified at seven levels throughout its septo-temporal extent (see [2]). Acidophilic Purkinje cells were counted in all sections through the cerebellum.

No significant differences between the two experimental groups were observed in any of the physiological variables measured during (Table 1) or after the period of status epilepticus. Fig. 1 shows the NA concentrations and the number of acidophilic, necrotic neurons in the cerebral cortex in the two groups 1 week after the epileptic insult. The 6-hydroxydopamine lesions caused a reduction of the NA levels in the frontal cortex by 94% (ranging from 89% to 98% in the individual hemispheres). The mean number of necrotic neurons in the cortex of sham-operated animals was found to be 22 (range 1-57) as counted in a coronal section through the parietal cortex at the level of the subfornical organ.The necrotic neurons were observed almost exclusively in layers III and IV, particularly in layer IV. Also in the NA-depleted animals the necrotic neurons were largely confined to these cortical layers. However, the 6-hydroxydopamine lesions caused a tripling ot the number of necrotic neurons as compared to sham operated rats, at the defined level of the cerebral cortex. The number of acidophilic neurons in the NA-depleted animals was 69 (14-134).

Necrotic neurons were quantified also in the hippocampus, caudoputamen, and cerebellum (Table 2). Very few necrotic neurons were found in these structures and no significant differences were found in the number of necrotic neurons between NA-depleted and sham-operated animals.

Table 1

Physiological parameters in the sham-operated and noradrenaline-lesioned animals during status epilepticus

	MABP mmHg	pO_2 mmHg	pCO_2 mmHg	pH	Temp ^oC
NA-lesioned n=8	111±7	110±11	39.1±1.3	7.31±0.02	37.2±0.2
sham-operated n=6	108±4	110±9	37.8±1.2	7.30±0.03	37.4±0.1

MABP= Mean arterial blood pressure. Values are given as mean±SEM

Table 2. Number of necrotic neurons in three different regions of sham-operated and noradrenaline-lesioned animals. Numbers are expressed as means (range).

	Sham-operated	NA-lesioned
Caudoputamen	2 (0-6)	2 (0-3)
Hippocampus	4 (0-6)	7 (0-48)
Cerebellum	74 (1-360)	20 (0-100)

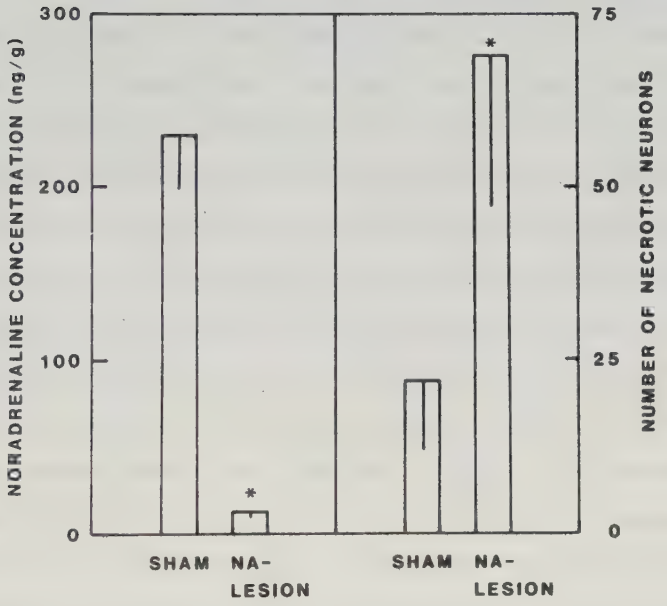

FIG 1.

NA concentration in the frontal cortex of sham-operated (12 hemispheres) and NA lesioned animals (16 hemispheres) (left panel) and number of necrotic neurons in the parietal cortex in the same animal groups (6 and 8 animals, respectively; right panel). Bars indicate mean - SEM. Statistical significance in differences was calculated using the non-parametric Mann-Whitney U-test. * denote $p \leq .05$

Our finding that removal of the noradrenergic input to the neocortex aggravates neuronal necrosis following status epilepticus implicates that the LC system has a protective action against epileptic brain damage. This further substantiates the hypothesis that transmitter-receptor interactions at excitatory and inhibitory synapses play important roles in the pathogenesis of selective neuronal necrosis in epilepsy, hypoglycemia and ischemia. Several experimental findings seem to indicate that enhanced excitatory neurotransmission at glutamatergic and/or aspartergic synapses causes postsynaptic neuronal necrosis in these conditions. For example, neuronal necrosis induced by anoxia in cell culture requires intact synaptic function and can be reproduced by addition of glutamate to the culture [15,16]. Furthermore, lesions of excitatory (presumably glutamatergic) inputs to striatum (from neocortex) and hippocampus (from entorhinal cortex) ameliorate neuronal necrosis in these areas in hypoglycemia and ischemia, respectively [20,21]. A protective effect against ischemic brain damage has also been reported with local intrahippocampal injection of a glutamate receptor antagonist [18].

In a previous study we have shown [3] that lesions of the forebrain projection from the LC increase the density of neuronal necrosis in the hippocampus and causes cell damage in the neocortex following complete cerebral ischemia. The protective action of the LC system in ischemia and status epilepticus, as observed in the present experiments, is most likely related to the well characterized inhibitory function of this system in cortical and hippocampal areas [8]. During status epilepticus the LC system is activated [4,9,10], which leads to accumulation of the second messenger cyclic AMP [9,10] and probably has a depressant effect on

neuronal excitability. In LC-lesioned animals the balance between excitatory and inhibitory receptor stimulation during status epilepticus is changed towards a predominance of excitatory synaptic mechanisms. In the neocortex this leads to an aggravation of neuronal necrosis following 60 min of status epilepticus.

The degree of cell damage in the hippocampus was not changed by the lesion. Although NA levels were not determined in the hippocampus it seems most likely that the lesion removed the major part of the dense noradrenergic innervation in this structure (cf.[10,12]). Our findings indicate that after 60 min status epilepticus, even in the absence of the inhibitory NA input, the hyperexcitation induced by release of excitatory amino acids is insufficient to cause significant neuronal necrosis in the hippocampus. The LC-system probably acts only as a modulator in the development of epileptic brain damage. Therefore, no effect of the NA depletion will be recorded unless a number of neurons are on the border of necrosis already in the non-NA-depleted animals. In the hippocampus this will probably occur after longer seizure periods than 60 min and then the NA depletion would possibly reveal a protective action of the LC system also in this region. The lack of effect of the LC lesion in the caudoputamen and cerebellum is consistent with the fact that the caudoputamen contains very few NA fibers [12] and that the NA input to the cerebellum is not severed by the lesion (unpublished observations).

The present data further add to the current interest for the LC system in the pathophysiology of epileptic seizures. In addition to a suppressant function in the generation or spread of seizures, this system also seems to protect against epileptic brain damage. These

effects, which may be viewed in the context of a general "stress-dampening" role of the LC system, may contribute to better understanding and treatments also of epilepsy in humans.

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Cerebral Metabolic Changes During and Following Fluorothyl-Induced Seizures in Ventilated Rats

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Abstract: The objective of the present study was to assess metabolic changes in the neocortex and hippocampus of well-oxygenated or moderately hypoxic rats in which fluorothyl-induced seizures were sustained for 5 or 20 min, or which were allowed recovery periods of 5, 15, or 45 min following cessation of 20-min seizure activity by withdrawal of the convulsant gas. Sustained fluorothyl-induced seizures were found to cause metabolic alterations qualitatively and quantitatively similar to those previously observed with other commonly used convulsants. Thus, although the phosphorylation state of the adenine nucleotide pool remained only moderately perturbed, if at all, there were decreases in tissue concentrations of phosphocreatine and glycogen, and increases in those of cyclic AMP, lactate, and pyruvate, with a calculated fall in intracellular pH of about 0.15 units and a rise in the cytoplasmic NADH/NAD^+ ratio. The enhanced metabolic rate was reflected in a marked reduction in the tissue-to-plasma glucose concentration ratio. Induced moderate hypoxia (arterial Po_2 40–50 mm Hg) had no metabolic effect after 5 min of seizures but moderately increased lactate concentrations after 20 min (from about 10 to about 15 $\mu mol \cdot g^{-1}$). On cessation of seizure discharge cyclic AMP and phosphocreatine concentrations normalized already within 5 min, whereas glycogen and lactate concentrations normalized more slowly. In the neocortex (but not the hippocampus) postepileptic tissue-to-plasma glucose concentration ratios rose above control, probably reflecting metabolic depression. The results suggest that intracellular pH promptly returned to control, and that postepileptic alkalosis developed. They also suggest that some elevation of the NADH/NAD^+ ratio persisted even after 45 min of recovery. **Key Words:** Fluorothyl—Seizures—Phosphocreatine—Metabolism—Neocortex—Hippocampus. **Folbergrová J. et al.** Cerebral metabolic changes during and following fluorothyl-induced seizures in ventilated rats. *J. Neurochem.* **44,** 1419–1426 (1985).

One of the principal goals of measurements of cerebral metabolic changes induced by repeated or sustained seizures is to delineate neurochemical events that may help to explain the neuronal damage, if incurred. It is suspected by many that complicating tissue hypoxia, due to arterial hypotension and/or hypoxia, is a contributory cause. For that reason, many studies of cerebral metabolic events have been carried out in artificially ventilated and well-oxygenated animals, i.e., under conditions of optimal tissue oxygenation (Duffy et al., 1975; Chapman et al., 1977; Blennow et al., 1977, 1979; Folbergrová et al., 1981, Siesjö et al., 1982; Ingvar et al., 1984). In general, such studies have shown that, even when sustained for up to 2 h, seizures cause only minimal perturbation of the phosphorylation state of the tissue adenine nucleotide pool, the chief metabolic effects encompassing glycogenolysis, stimulation of glycolysis with increases in tissue concentrations of lactate and pyruvate, reduction of the cytoplasmic NADH/NAD^+ system, increases in cyclic nucleotide concentrations, and accumulation of free fatty acids (FFAs). With generalized seizures cerebral metabolic rate is increased primarily in neocortical and limbic areas (Siesjö and Abdul-Rahman, 1979; Ingvar and Siesjö, 1983). As a corollary, perturbation of cerebral energy state is most conspicuous in these structures, assumed to be vulnerable to epileptic insults, and less pronounced in "resistant" structures such as the cerebellum (Folbergrová et al., 1981; Siesjö et al., 1982; for comparable data on cerebral metabolic rates and blood flow, see Horton et al., 1980; Ingvar and Siesjö, 1983).

Studies of the relationship between metabolic events and development of irreversible brain

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Abbreviation used: PCr, phosphocreatine.

damage have been hampered by the lack of suitable recovery models, allowing assessment of the final cell damage incurred. This is because general convulsants such as bicuculline and pentylenetetrazol damage the cardiovascular system to the extent that recovery, induced by, e.g., diazepam, becomes compromised (see, e.g., Söderfeldt et al., 1983). In this respect, the convulsant gas fluorothyl offers certain advantages. It has the unique feature of allowing seizures to be arrested without the use of anticonvulsant drugs, provided the seizures have not lasted for more than 30 min. The compound has previously been used to assess cerebral metabolic changes during short-lasting seizures (Sacktor et al., 1966; Howse et al., 1974; Duffy et al., 1975). We used the fluorothyl model in ventilated rats to assess metabolic and circulatory events during seizures of 20 min duration, as well as in the recovery period following the spontaneous arrest of seizure activity. We have also shown that seizures lasting less than 30 min failed to induce permanent brain damage, as assessed by histopathologic techniques after 1 week of survival, but that longer seizure periods gave rise to lesions in the pallidoreticularis system, in the neocortex and the hippocampus (chiefly the CA4 sector) (Nevander et al., 1984; G. Nevander, M. Ingvar, R. Auer, and B. K. Siesjö, in preparation).

Our objectives were three. First, we wished to study whether fluorothyl induces metabolic changes in the neocortex and hippocampus similar to those observed with bicuculline and pentylenetetrazol. Second, we explored the effect of a reduced arterial P_{O_2} (down to 40–50 mm Hg) during the seizure period. Third, we wanted to characterize events during the recovery period. In subsequent publications, we intend to correlate the changes observed to the local metabolic rate/blood flow couple and to changes in extra- and intracellular pH.

MATERIALS AND METHODS

Materials

Male Wistar rats of an S.P.F. strain (Møllegaard's Avlslaboratorium, Copenhagen, Denmark), weighing 310–400 g, were used. The animals had free access to pellet food (Astra-Ewos, Södertälje, Sweden) and tap water until the time of experiments. Fluorothyl (hexafluorodiethyl ether) was supplied by Armageddon (Armageddon, OH). Atropine sulfate and suxamethonium chloride were obtained from ACO (Stockholm, Sweden) and phentolamine was supplied by Ciba-Geigy (Basel, Switzerland). Coenzymes and substrates for enzymatic assays were from Sigma Chemical (St. Louis, MO) except lactate dehydrogenase, which was from Millipore (Bedford, MA). All other enzymes were from Boehringer and Sohn (Darmstadt, West Germany). Cyclic AMP kits were obtained from the Radiochemical Centre (Amersham, U.K.).

Operative and sampling techniques

The animals were anesthetized with 3% halothane and 65–70% N_2O. When unresponsive, they were tracheotomized and ventilated on 1% halothane in 65–70% N_2O and 30–35% O_2. Femoral arterial and venous catheters were inserted for infusions, sampling of arterial blood, and blood pressure recording. Body temperature was monitored in the rectum. Gold-plated copper bolts were inserted in the skull bone and connected to an Elema EEG machine to provide bipolar EEG records. A skin incision was placed over the calvarium to accommodate a plastic funnel for later freezing of the brain in situ (Pontén et al., 1973). After completion of the operative procedures the halothane supply was discontinued, and the animals were ventilated on the N_2O-O_2 mixture for about 30 min, body temperature being adjusted to 37°C and arterial P_{O_2} and P_{CO_2} to values of >90 mm Hg and 30–40 mm Hg, respectively. Atropine (0.1 mg · kg^{-1}) was given intravenously to prevent problems with the airway.

Induction and maintenance of seizures

Seizures were induced by adding 80 μl of the convulsant gas to a rebreathing system connected to the inlet of the respirator. Two procedures were instituted to curtail the ictal increase in blood pressure. First 3–4 ml of blood was withdrawn prior to addition of the convulsant. Second, 1–2 min before the fluorothyl was added a slow infusion of phentolamine was given intravenously (0.05 ml · min^{-1} of a 0.8 mg · ml^{-1} solution). The infusion was discontinued after that, the blood pressure had decreased to 100–120 mm Hg, and at this point the fluorothyl was injected into the rebreathing system. If required to maintain pressure at these levels, the shed blood was reinfused. If the burst-discharge pattern became attenuated, an additional dose of fluorothyl (20 μl) was given. In all animals, a burst-suppression pattern could be sustained for the duration of the seizure period.

Two series of animals were studied. In one, sufficient oxygen was supplied to maintain arterial P_{O_2} >100 mm Hg during the seizures. In the other, arterial P_{O_2} was reduced to 40–50 mm Hg at the onset of seizures and maintained at that level for the remainder of the seizure period. In each series, control animals (n = 4) and seizure groups (n = 4) were studied after 5 and 20 min of seizure activity, as well as after 5, 15, and 45 min of recovery after a seizure period of 20 min. In all experiments but two, seizures could be arrested promptly by discontinuation of fluorothyl supply (see below). Arterial samples for measurements of pH and blood gases as well as of glucose, lactate, and pyruvate concentrations, were collected at least twice following seizure induction, the last sample being taken just prior to freezing of the brain in situ.

Methods and analytical techniques

Blood gases and pH were measured with microelectrodes, operated at 37°C with due corrections for body temperature (blood gases: Eschweiler and Co., Kiel, West Germany; pH: Radiometer, Copenhagen, Denmark). Tissue metabolites were measured on 20–30-mg samples from the fronto-parietal neocortex and from the hippocampal formation. The samples were dissected at −22°C, weighed, and extracted with HCl-methanol, followed by perchloric acid at 0°C. The subsequent handling of the extracts and the measurements of substrates with

enzymatic-fluorometric techniques have been described in detail previously (see Folbergrová et al., 1981). Similar techniques were used to measure glucose, lactate, and pyruvate in arterial blood. A similar extraction technique was used to measure cyclic AMP with a protein-binding technique.

Calculations

The adenylate energy charge was calculated according to Atkinson (1968). Changes in the cytoplasmic NADH/NAD^+ ratio were calculated from the lactate dehydrogenase equilibrium, and changes in intracellular pH (pH_i) from the creatine kinase equilibrium, as described previously (Siesjö et al., 1972; Chapman et al., 1977). For calculation of statistical differences between groups, we used analysis of variance (ANOVA), followed by Newman-Keul's test. The control groups for the nonhypoxic and hypoxic animals (which were treated identically) were pooled to comprise a group of eight control animals. Differences between control-seizure groups and control-recovery groups were calculated separately.

RESULTS

We describe in turn EEG changes during and following seizures, physiological parameters, blood substrates, and changes in tissue metabolites.

EEG patterns

Figure 1 illustrates representative changes in EEG pattern at the onset of seizure discharge; after 5 and 20 min of seizures; following the discontinuation of fluorothyl supply; as well as after 5, 15, and 45 min of recovery. As observed, an epileptic pattern of spikes and waves appeared about 10–30 s following fluorothyl administration. The subsequent pattern observed was similar to that described for bicuculline-induced seizures (see Chapman et al., 1977), and a continuous burst and suppression pattern prevailed in the period 5–20 min. Following discontinuation of fluorothyl supply the epileptic bursts disappeared within 1–2 min, to be followed by EEG depression. Two animals, in which the seizure activity was maintained after discontinuation of fluorothyl, received 15 mg/kg of sodium pentothal to stop the seizure activity. In no parameter measured was it possible to distinguish these two animals from the others in the same groups. Subsequently, a low-amplitude slow-wave EEG activity began to appear and, after 45 min, the pattern was close to control, albeit with intermingled slow-wave activity.

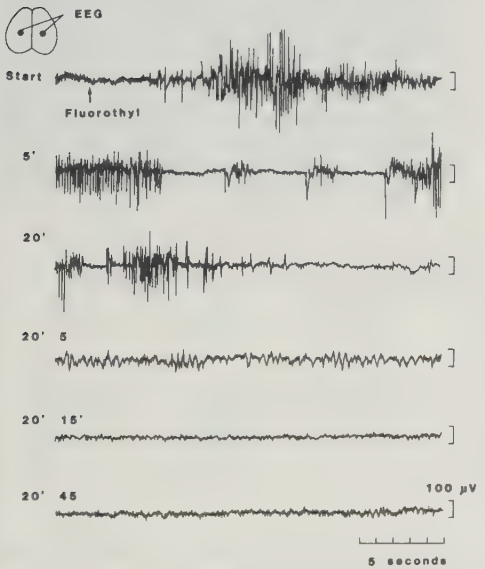

FIG. 1. Changes in EEG activity during and following fluorothyl-induced seizures. The upper three records illustrate EEG changes at seizure induction, as well as after 5 and 20 min of seizures, whereas the lower three tracings illustrate EEG after 5, 15, and 45 min of recovery, following 20 min of seizures.

Physiological parameters and blood substrates

As Table 1 shows, all groups had body temperatures close to 37°C, and arterial blood pressure >115 mm Hg (no animal had a blood pressure <110 mm Hg). All nonhypoxic animals had arterial Po_2 values >90 mm Hg whereas the hypoxic ones (5 and 20 min of seizures) had Po_2 values in the 30–50 mm Hg range. Pco_2 values were in the range 30–40 mm Hg (one previously hypoxic animal with 5-min recovery had a Pco_2 of 48 mm Hg). In both series plasma pH fell moderately during seizures, but recovery values were close to control.

Blood glucose, lactate, and pyruvate concentrations in control animals were as expected (data not given). Thus, in the fed animals studied blood glucose concentrations exceeded 8 $\mu mol \cdot g^{-1}$whereas lactate concentrations were <1.5 $\mu mol \cdot g^{-1}$, with a lactate/pyruvate ratio of close to 10. In nonhypoxic animals, seizures were accompanied by an increase in glucose concentration approaching 200% of control whereas lactate concentrations rose four- to sixfold, with a corresponding rise in the lactate/pyruvate ratio. In hypoxic animals, blood glucose concentrations during seizures reached 200% of control; the increases in lactate concentration and the lactate/pyruvate ratio were somewhat more pronounced. In both series, though, all substrate concentrations were close to control after 45 min of recovery. In all probability, the hyperglycemia and the accumulation of lactate and pyruvate reflect the effects of seizure-induced sympatho-adrenal activation which were only partly curbed by the small doses of phentolamine used.

Cerebral metabolic state: neocortex

Metabolic changes in neocortex are shown in

TABLE 1. *Physiological variables of the experimental groups prior to freezing of the tissue in situ*

		Control (n = 8)	5-min seizures (n = 4)	20-min seizures (n = 4)	5-min recovery (n = 4)	15-min recovery (n = 4)	45-min recovery (n = 4)
MABP	No hypoxia	148 ± 13	135 ± 11	128 ± 5	130 ± 9	116 ± 6	125 ± 4
(mm Hg)	Hypoxia		114 ± 3	120 ± 17	150 ± 18	129 ± 15	134 ± 11
Po_2	No hypoxia	102 ± 7	>130[a]	>130[a]	126 ± 24	115 ± 24	119 ± 8
(mm Hg)	Hypoxia		43 ± 11	43 ± 7	112 ± 10	112 ± 12	115 ± 4
Pco_2	No hypoxia	37.4 ± 1.7	36.2 ± 2.3	37.5 ± 4.5	37.7 ± 1.4	35.8 ± 4.1	38.8 ± 2.8
(mm Hg)	Hypoxia		32.1 ± 4.3	36.0 ± 0.9	39.5 ± 6.2	37.1 ± 2.5	40.1 ± 3.7
pH	No hypoxia	7.46 ± 0.03	7.37 ± 0.03	7.30 ± 0.02	7.29 ± 0.10	7.41 ± 0.06	7.39 ± 0.07
	Hypoxia		7.36 ± 0.05	7.29 ± 0.05	7.29 ± 0.10	7.35 ± 0.08	7.39 ± 0.07
Temperature	No hypoxia	37.2 ± 0.4	36.8 ± 0.3	37.2 ± 0.2	37.4 ± 0.6	37.1 ± 0.7	37.0 ± 0.4
(°C)	Hypoxia		36.4 ± 0.2	36.9 ± 0.7	37 ± 0.9	37.3 ± 0.1	37.1 ± 0.2

Values are given as means ± SEM, except in groups marked with *a*, where altogether three animals had Po_2 values exceeding the range of the blood gas measurement. In these groups the values are given as the minimum value recorded.

Table 2. The seizures did not significantly alter ATP concentrations, or the sum of adenine nucleotides. However, in both normoxic and hypoxic animals seizures were accompanied by increased mean values for ADP and AMP, and by decreased values for the calculated energy charge. These changes did not reach statistical significance. However, when the control materials of the two series and the 5- and 20-min values for each series were pooled, statistical treatment supported the assumption that seizures caused a small perturbation of the phosphorylation state of the adenine nucleotide pool. It seems clear, though, that added hypoxia did not affect the energy charge, and that the recovery values did not differ from those measured during seizures. We conclude that adenine nucleotide concentrations were minimally affected.

All other substrates changed during seizures. In both series, cyclic AMP concentration increased by 1 $\mu mol \cdot kg^{-1}$ after 5 and by 0.3–0.4 $\mu mol \cdot kg^{-1}$ after 20 min of seizures, with normalization already after 5 min of recovery. The phosphocreatine (PCr) concentration was reduced during seizures, progressively so in the hypoxic animals, and normalized already 5 min after termination of seizure discharge. In fact, values recorded during recovery exceeded those of controls (see below). Changes in creatine concentrations were not simply reciprocal of those in PCr, as the sum of creatine and PCr was elevated in the seizure and recovery groups (see below).

The most conspicuous seizure-induced alterations affected the glycolytic substrates. The glycogen concentration was markedly reduced during seizures in normoxic and hypoxic animals, but was virtually normalized after 45 min of recovery. Glucose concentrations did not change during seizures but rose during recovery (see below). After 5 min of seizures, lactate concentrations in normoxic animals reached the "ceiling" values of 8–10 $\mu mol \cdot g^{-1}$ previously recorded in bicuculline-induced seizures (Chapman et al., 1977). After 5 min of seizures values in hypoxic animals were identical but the results indicate some further accumulation after 20 min. Pyruvate rose as well; however, since the increase in lactate was proportionally larger the

TABLE 2a. *Labile energy metabolites in the cerebral cortex during and after seizures induced with fluorothyl in well-oxygenated animals*

	Control (n = 8)	5-min seizures (n = 4)	20-min seizures (n = 4)	5-min recovery (n = 4)	15-min recovery (n = 4)	45-min recovery (n = 4)
PCr	4.19 ± 0.08	3.18 ± 0.16*	3.30 ± 0.34	4.48 ± 0.09	4.53 ± 0.07	4.63 ± 0.07
Cr	5.93 ± 0.07	7.87 ± 0.26*	7.91 ± 0.29*	6.27 ± 0.16	6.51 ± 0.06*	6.61 ± 0.08*
ATP	2.94 ± 0.02	2.94 ± 0.06	2.99 ± 0.05	2.95 ± 0.03	2.96 ± 0.06	3.03 ± 0.04
ADP	0.274 ± 0.009	0.307 ± 0.014	0.320 ± 0.023	0.281 ± 0.023	0.303 ± 0.015	0.298 ± 0.009
AMP	0.057 ± 0.002	0.066 ± 0.004	0.070 ± 0.004	0.071 ± 0.004	0.066 ± 0.002	0.077 ± 0.010
Energy charge	0.941 ± 0.001	0.934 ± 0.002	0.931 ± 0.003	0.936 ± 0.002	0.935 ± 0.001	0.934 ± 0.002*
Glucose	2.91 ± 0.18	2.01 ± 0.02	2.64 ± 0.26	6.50 ± 0.73	5.69 ± 0.43*	4.13 ± 0.69*
Glycogen	2.15 ± 0.19	0.86 ± 0.08*	0.69 ± 0.08*	1.17 ± 0.44	1.16 ± 0.18	2.15 ± 0.24
Lactate	1.75 ± 0.12	10.50 ± 0.50*	8.83 ± 0.35*	4.54 ± 1.13*	3.57 ± 0.41	2.13 ± 0.12
Pyruvate	0.128 ± 0.008	0.188 ± 0.017	0.178 ± 0.016	0.163 ± 0.028	0.162 ± 0.019	0.116 ± 0.008
Lactate/pyruvate	13.7 ± 0.3	57.2 ± 5.6*	50.5 ± 2.8*	26.8 ± 2.0*	22.0 ± 0.7*	18.6 ± 1.3
Cyclic AMP	1.58 ± 0.06	2.60 ± 0.14*	1.98 ± 0.07	1.64 ± 0.12	1.58 ± 0.11	1.61 ± 0.07

Values are given as means ± SEM in μmol/g, except values for cyclic AMP which are given in nmol/g.
* $p < 0.01$ from control.

TABLE 2b. *Labile energy metabolites in the cerebral cortex during and after seizures induced with fluorothyl in animals with moderate hypoxia*

	Control (n = 8)	5-min seizures (n = 4)	20-min seizures (n = 4)	5-min recovery (n = 4)	15-min recovery (n = 4)	45-min recovery (n = 4)
PCr	4.19 ± 0.08	3.47 ± 0.44	2.44 ± 0.27*	4.49 ± 0.18	4.40 ± 0.06	4.84 ± 0.10*
Cr	5.93 ± 0.07	7.63 ± 0.44*	8.39 ± 0.33*	6.50 ± 0.19	6.70 ± 0.16*	6.48 ± 0.21
ATP	2.94 ± 0.02	2.76 ± 0.05	2.83 ± 0.05	2.86 ± 0.05	2.85 ± 0.08	2.97 ± 0.07
ADP	0.274 ± 0.009	0.353 ± 0.054	0.316 ± 0.016	0.305 ± 0.015	0.309 ± 0.010	0.309 ± 0.010
AMP	0.057 ± 0.002	0.071 ± 0.003	0.067 ± 0.008	0.065 ± 0.009	0.067 ± 0.005	0.060 ± 0.004
Energy charge	0.941 ± 0.001	0.922 ± 0.008	0.930 ± 0.002	0.932 ± 0.001	0.931 ± 0.002*	0.935 ± 0.001
Glucose	2.91 ± 0.18	2.45 ± 0.28	3.79 ± 0.53	7.62 ± 0.52*	5.70 ± 0.82*	4.00 ± 0.51
Glycogen	2.15 ± 0.19	0.72 ± 0.10*	0.58 ± 0.06*	0.75 ± 0.08*	1.07 ± 0.13*	1.79 ± 0.05
Lactate	1.75 ± 0.12	10.89 ± 0.64*	13.56 ± 0.54*	6.48 ± 0.77*	3.64 ± 0.58*	1.49 ± 0.19
Pyruvate	0.128 ± 0.008	0.184 ± 0.005*	0.192 ± 0.005*	0.232 ± 0.028*	0.165 ± 0.013	0.096 ± 0.003
Lactate/pyruvate	13.7 ± 0.3	59.8 ± 5.0*	70.6 ± 1.8*	28.0 ± 1.1*	21.6 ± 2.1*	15.5 ± 1.7
Cyclic AMP	1.58 ± 0.06	2.56 ± 0.10*	1.95 ± 0.05*	1.66 ± 0.06	1.62 ± 0.07	1.46 ± 0.08

Values are given as means ± SEM in μmol/g, except values for cyclic AMP which are given in nmol/g. During the 20-min seizure period the animals were hypoxic.
* $p < 0.01$ from control.

lactate/pyruvate ratio increased three- to fivefold. Cessation of seizure discharge brought about a relatively slow recovery. Thus, lactate concentrations were still elevated after 15 min of recovery, and so was the lactate/pyruvate ratio.

Cerebral metabolic state: hippocampus

Metabolic changes in the hippocampus were qualitatively and quantitatively similar to those observed in the neocortex. Table 3, therefore, only gives data on normoxic animals. Notably, in this structure changes in creatine concentration were reciprocal to those in PCr, and no evidence was obtained that the sum of the two increased during seizures. As observed, changes in glycogen, lactate, and pyruvate concentrations were virtually identical to those obtained in the neocortex (see Table 2 above). After 5 and 20 min of seizures in the hypoxic animals, lactate concentrations (means ± SEM) were 10.29 ± 0.49 and 14.35 ± 0.51 μmol $\cdot g^{-1}$, respectively, i.e., very similar to those measured in the neocortex. However, the postinsult increase in tissue glucose concentration was less conspicuous in the normoxic animals, but present in the hypoxic ones (see below).

Tissue-to-plasma glucose concentration ratios

It was previously shown that seizures lead to a reduction in the tissue-to-plasma glucose concentration ratio, probably reflecting the increased glucose utilization (Duffy et al., 1975; Chapman et al., 1977; Folbergrová et al., 1981; Ingvar and Siesjö, 1985). Figure 2 demonstrates that this also occurred during fluorothyl seizures. In the neocortex, but not the hippocampus, cessation of seizure discharge caused the glucose ratio to rise above control. In the former structure, therefore, postinsult accumulation of glucose was due both to the lingering increase in plasma glucose concentration and to a rise in the tissue-to-plasma glucose concentration ratio.

Intracellular pH and cytoplasmic NADH/NAD$^+$ ratios

Changes in pH_i were calculated on the assumption that these are reflected in the creatine kinase

TABLE 3. *Labile energy metabolites in the hippocampus during and after seizures induced with fluorothyl*

	Control (N = 8)	5-min seizures (n = 4)	20-min seizures (n = 4)	5-min recovery (n = 4)	15-min recovery (n = 4)	45-min recovery (n = 4)
PCr	4.93 ± 0.07	3.81 ± 0.15*	3.84 ± 0.33*	4.77 ± 0.13	4.65 ± 0.11	4.93 ± 0.04
Cr	6.51 ± 0.09	7.57 ± 0.20	7.63 ± 0.29	6.64 ± 0.21	7.38 ± 0.09*	6.76 ± 0.17
ATP	2.98 ± 0.04	2.82 ± 0.02	2.79 ± 0.06	2.87 ± 0.02	2.90 ± 0.05	2.95 ± 0.02
ADP	0.310 ± 0.015	0.337 ± 0.016	0.341 ± 0.018	0.326 ± 0.021	0.342 ± 0.021	0.325 ± 0.013
AMP	0.069 ± 0.003	0.076 ± 0.007	0.076 ± 0.006	0.072 ± 0.004	0.079 ± 0.006	0.073 ± 0.002
Energy charge	0.933 ± 0.002	0.925 ± 0.004	0.923 ± 0.001	0.928 ± 0.002	0.924 ± 0.004	0.929 ± 0.001
Glucose	3.19 ± 0.21	1.75 ± 0.22*	2.03 ± 0.18*	4.74 ± 0.55	4.39 ± 0.48	3.62 ± 0.57
Glycogen	3.05 ± 0.16	1.37 ± 0.07*	0.70 ± 0.08*	1.00 ± 0.20*	1.03 ± 0.14*	2.30 ± 0.32
Lactate	1.57 ± 0.14	10.16 ± 0.48*	8.85 ± 0.73*	4.74 ± 0.82*	5.70 ± 0.35*	2.66 ± 0.44
Pyruvate	0.118 ± 0.006	0.173 ± 0.013*	0.155 ± 0.015	0.195 ± 0.026	0.229 ± 0.021*	0.151 ± 0.014
Lactate/pyruvate	13.3 ± 1.2	59.6 ± 3.9*	57.3 ± 2.4*	24.1 ± 1.5*	25.0 ± 0.84*	17.7 ± 2.8
Cyclic AMP	1.01 ± 0.05	1.50 ± 0.14*	1.18 ± 0.05	0.99 ± 0.10	0.95 ± 0.08	0.94 ± 0.04

Values are given as means ± SEM in μmol/g, except values for cyclic AMP which are given in nmol/g.
* $p < 0.01$ from control.

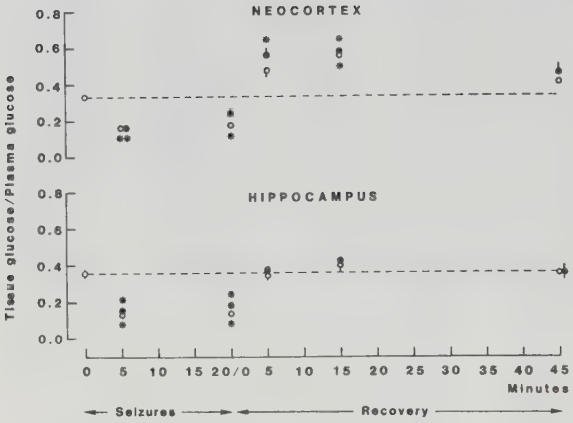

FIG. 2. Tissue-to-plasma glucose concentration ratio for neocortical and hippocampal samples during and following fluorothyl-induced seizures. (○), Well-oxygenated animals; (●), animals subjected to mild hypoxia during the seizure period. Values are given as means ± SEM. *Difference from control ($p < 0.01$).

equilibrium (see Materials and Methods). For the two control series, the PCr, Cr, ATP, and ADP concentrations were used to derive a provisional K_{eq} for this reaction, assuming a control pH_i of 7.0, and the K_{eq} derived was then used to calculate pH_i during seizures and in the recovery period (see Siesjö et al., 1972; Chapman et al., 1977). Since changes in extra- and intracellular pH will be the subject of a separate communication (Siesjö et al., 1985) only the values obtained in the neocortex of normoxic animals are given in Fig. 3. Three points can be made. First, the decrease in pH_i during seizures approximates 0.15 units. Second, pH_i had normalized already 5 min after cessation of seizure discharge, and the values derived suggest that a moderate postinsult alkalosis occurred. Third, using these values and the lactate/pyruvate ratios it emerged that the cytoplasmic NADH/NAD^+ ratios rose during seizures, and did not approach control values until after 45 min of recovery.

DISCUSSION

As stated in the introductory section, one of the main objectives of the present study was to study metabolic changes both during ongoing seizures, and during recovery induced by withdrawal of the convulsant in a model that obviated the use of anticonvulsant drugs. The results obtained provide answers to the questions posed. In discussing these

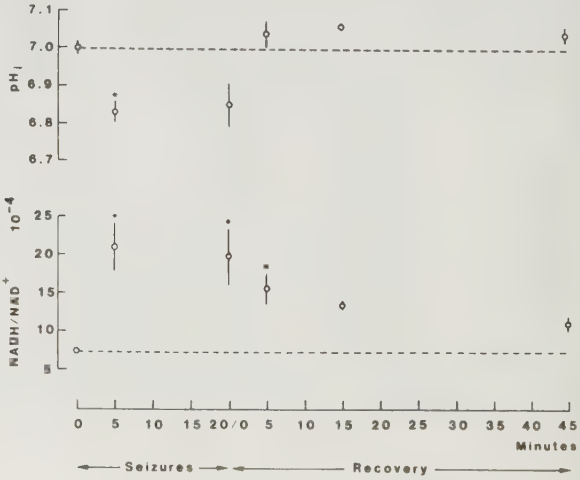

FIG. 3 Intracellular pH (pH_i) and NADH/NAD^+ ratios in neocortex during fluorothyl-induced seizures in well-oxygenated animals. Values are given as means ± SEM. *Difference from control ($p < 0.01$).

results, we wish to recall that the maximal seizure period employed (20 min) does not lead to permanent neuronal damage (Nevander et al., 1984).

It is clear that seizures induced by fluorothyl are accompanied by cerebral metabolic changes that are qualitatively and quantitatively similar to those previously recorded during seizures induced by bicuculline (Chapman et al., 1977; Blennow et al., 1977, 1979, Folbergrová et al., 1981) and pentylenetetrazol (Duffy et al., 1975; Ingvar et al., 1984), as well as to those reported for repeated administration of fluorothyl (Duffy et al., 1975; see also Sacktor et al., 1966). These changes encompass reduced tissue concentrations of PCr and glycogen and raised concentrations of cyclic AMP, lactate, and pyruvate, with a reduction in pH_i and increase in the cytoplasmic NADH/NAD^+ ratio (cf. Howse et al., 1974; Howse and Duffy, 1975; Chapman et al., 1977). All these changes must reflect the enhanced cerebral metabolic rate. We also recall that the reduction in the tissue-to-plasma glucose concentration ratio should have the same cause (see Ingvar and Siesjö, 1985). The present results reinforce one major finding in ventilated and well-oxygenated animals: following an initial perturbation (Chapman et al., 1977) the phosphorylation state of the adenine nucleotide pool remains close to control, and overt depletion of tissue ATP stores does not occur.

It was previously shown that if seizures are induced in moderately hypoxic animals the major metabolic alteration is an exaggeration of the tissue lactic acidosis (Blennow et al., 1977). The present results demonstrate that hypoxia of that magnitude does not elevate lactate levels after 5 min of seizures, and only a moderate exaggeration of the lactic acidosis is observed after 20 min. Since the previous data (Blennow et al., 1977) pertained to 2 h of seizure activity it seems clear that the effect of the added hypoxia occurs gradually during the seizures, and that marked acidosis does not develop until seizures are sustained for more than 20 min.

Previous recovery studies concerned seizures of relatively short duration, and the recovery periods studied were also rather short. The present results give information on recovery events following cessation of seizures that had been lasting continuously for 20 min and recovery was followed for 45 min. It could be shown that the pattern of normalization of metabolites was similar to that described in some previous reports (Howse et al., 1974; Folbergrová, 1974, 1977). Thus, some variables normalize already within the first 5 min, e.g., cyclic AMP and the PCr concentrations, whereas resynthesis of glycogen and disappearance of the lactate accumulated occur gradually. Two findings are of special interest. First, in the neocortex, but not in the hippocampus, tissue-to-plasma glucose concentration ratios rose above control. It is tempting to conclude that this rise reflects postepileptic depression of metabolism; by inference, one must then postulate that such a depression does not affect the hippocampal formation. However, this conclusion must remain speculative until confirmed by direct measurements. Second, the results obtained suggest that normalization of pH_i occurs already within the first postepileptic 5-min period, and that a moderate alkalosis then develops. We wish to emphasize that calculation of pH_i from the creatine kinase equilibrium is tentative because it rests on the unvalidated assumption that equilibrium is maintained during seizures, and since further uncertainty is introduced by unexplained variations in the sum of PCr and Cr. However, as will be reported in the accompanying publication (Siesjö et al., 1985), such an alkalosis can be documented by more direct methods for assessing pH_i. It is thus akin to the postischemic alkalosis recently reported (Mabe et al., 1983).

As mentioned in the introductory section, the fluorothyl model of seizure induction has been adopted for studies of long-term recovery, and for assessment of the brain damage incurred by defined periods of sustained seizures (Nevander et al., 1984). In view of the present results, demonstrating that fluorothyl-induced seizures are accompanied by cerebral metabolic changes similar to those induced by other, commonly used convulsants, it would seem that results obtained with the fluorothyl model should be generally valid for generalized seizures.

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LOCAL CEREBRAL GLUCOSE UTILIZATION AND BLOOD FLOW IN THE RAT DURING AND FOLLOWING FLUROTHYL-INDUCED STATUS EPILEPTICUS.

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Summary: The main objective of the present study was delineate local metabolic rate and blood flow in the postictal period following flurothyl-induced seizures in rats. Local cerebral glucose utilization (l-CMRgl) and local cerberal blood flow (l-CBF) were measured with auto radiographic techniques.

Following the arrest of seizures, after a 20 min period of status epilepticus, CMRgl and CBF were decreased as compared to control. The depression of both variables was most pronounced in the neocortex and still persisted 45 minutes after the seizure arrest (30%-80 of control). The local coupling between metabolism and blood flow was disturbed 5 min after seizure arrest, but after 45 min, the coupling had essentially returned.

During the seizures, there was an increase in both l-CMRgl and l-CBF of the same magnitude and distribution as has previously been reported for bicuculline seizures in rats. It was concluded that status epilepticus induced in ventilated animals is the same whether induced by flurothyl or bicuculline.

Previous studies with flurothyl seizures have shown that the extracellular pH is reduced in the parietal cortex during and following status epilepticus. Thus, changes in the neocortical blood flow do not correlate with changes in extracellular pH.

Key words: Local cerebral blood flow - local cerebral glucose utilization -status epilepticus - regulation of cerebral blood flow -post-ictal depression.

It has been known for long that the cerebral metabolic rate and blood flow are increased during seizures (Roy and Sherrington 1890, Penfield and Jasper 1953, Plum et al 1968, Meldrum and Nilsson 1976, Borgström et al 1976). Data for global changes in the post epileptic period concerns mostly CBF in epileptic patients (Grant et al 1947, Kety et al 1948, Meyer et al 1966, Magistretti et al 1983). No report exists on regional cerebral metabolic rate and blood flow during this period.

The establishment of a model for status epilepticus with possibilities for recovery (Nevander et al 1985) now allows the study of the local metabolism and circulation in the post ictal phase after extended seizure periods. Thus it is possible to address the question whether status epilepticus, like ischemia and hypoglycemia (Nordström and Rehncrona 1977, Abdul Rahman and Siesjö 1981, Pulsinelli et al 1982), is followed by a period of metabolic depression and decreased perfusion. We used the deoxyglucose method to study the severeness, duration and regional distribution of the metabolic depression following 20 min of status epilepticus.

In a parallell set of animals local cerebral blood flow was assessed by use of 14C-iodo-antipyrine as the diffusible tracer. This was undertaken in order to study if the postictal depression of flow was correlated in magnitude and distribution to the changes in the metabolic rate.

By including a group of rats studied during seizures we wanted to find out whether a correlation exists between regions with a marked increase in the metabolic rate during seizures and those regions with the most marked depression in the post-ictal period. The ictal group

also allows a comparison of the metabolic pattern during flurothyl seizures with previous data obtained with other convulsants (Ingvar and Siesjö 1983).

MATERIALS AND METHODS:

Experimental groups: Local cerebral glucose utilization was studied in controls (n=5), and in animals with seizures where the isotope was allowed to circulate between 5 min to 50 min of seizure activity (n=3). Metabolic rate was studied in groups where the isotope was injected 5 min (n=4) and 45 min (n=4), respectively, following the spontaneous arrest of a 20 min seizure period. Local cerebral blood flow was measured in controls (n=6), in animals with 20 min status epilepticus (n=6) and in animals with 20 min seizure activity followed by either 5 or 45 min recocery after seizure arrest (n=7 in each).

Chemicals: ^{14}C-2-d-Deoxyglucose (New Englnad Nuclear, Boston, Mass., USA) was delivered in sterile water and diluted with Krebs solution. ^{14}C-Iodo-antipyrine (New England Nuclear, Boston, Mass., USA) was delivered in ethanol solution. Immediately prior to each experiment the ethanol was evaporated and the isotope was redissolved in Krebs solution. Flurothyl (Hexafluorodiethylether) was supplied by Armageddon Inc (Armageddon, Ohio, USA). Phentolamine (Ciba Geigy, Basel, Switzerland), atropine and suxamethonium chloride (Aco, Stockholm, Sweden) were diluted in Krebs solution before use.

Experimental procedures: Recent articles from our laboratory describe in more detail the model used (Folbergrova et al 1985, Nevander et al 1985). In short, fed male rats (315-415g) of an SPF strain (Møllegaard, Copenhagen, Denmark) were anesthetized in 3% halothane in a mixture of nitrous oxide and oxygen (2/1). When

unresponsive, they were tracheotomized and ventilated on a Starling type ventilator (Braun, Melsungen, FRG). Intermittent doses of suxamethonium chloride (3mg/kg of body weight) were used for immobilization. The halothane concentration was lowered to 0.7-1% for the rest of the operative procedures. Femoral artery catheters were inserted to allow monitoring of blood gases (Eschweiler and Co, Kiel, FRG), pH (Radiometer, Copenhagen, Denmark), plasma glucose (Beckman Instruments Inc, Fullerton, CA., USA) and blood pressure (Siemens Elema, Stockholm, Sweden). A femoral vein was catheterized to allow infusion of drugs and isotopes. The blood sampling for the determination of the arterial concentration of the isotope during the infusion was made from a brachial artery (see Dahlgren et al 1981). A one channel bipolar EEG was monitored from the frontoparietal region (Siemens Elema, Stockholm, Sweden). Atropine (0.05-0.1 mg/kg) was given to avoid airway problems.

The halothane supply was then discontinued, and the animals were left to eliminate the halothane for at least 30 min, during which time the pO_2 was adjusted to >90 mmHg and pCO_2 was set to between 35 and 40 mmHg. The body temperature was maintained close to 37°C. These parameters were kept within the ranges given above for the rest of the experiment.

The animals were given phentolamine iv (0.05 ml/min of a 0.8mg/ml solution) and 3-4 ml blood were withdrawn immediately prior to the start of the seizure activity, in order to avoid the ictal increase in blood pressure. When the animals had reached a blood pressure of 90-100 mmHg, they were connected to a rebreathing system, and 80 µl of flurothyl was injected directly into the system through the wall of the tubing. If necessary to maintain constant seizure activity, as

monitored on the EEG, supplementary doses of flurothyl (up to 50 µl totally) were injected into the rebreathing system. In order to maintain the blood pressure over 100mm Hg, the shed blood was reinfused. Some animals also required additional donor blood (up to 6 ml).

Recovery was instigated by connection of the animal to a normal open breathing system. In all animals, seizures ceased spontaneously when the flurothyl supply was discontinued.

Measurement of local cerebral glucose utilization (Sokoloff et al 1977) was performed as previously described (Ingvar and Siesjö 1983). The radioactive deoxyglucose was infused over 30 sec and allowed to circulate for 45 min before the animal was decapitated. 14 timed arterial samples were taken to allow determination of the radioactivity and glucose concentration in the plasma over time. Simultaneously with the last blood sample the animal was decapitated and the brain was rapidly removed and frozen in chilled isopenthane. The brain was sub-serially sectioned at -12-15°C. The slides then exposed a film (Kodak SB-5) for a week together with a precalibrated set of radioactive methylmethacrylate standards. The optical density of the film was manually evaluated by use of a densitometer with an aperture of 1 mm (Macbeth, TD 501, Newburgh, NY, USA). Measurement of the local cerebral blood flow was made autoradiographically as previously described (Ingvar and Siesjö 1983). The freely diffusible ^{14}C-iodoantipyrine was infused over 20 (seizure group) or 45 sec (control and recovery groups). During this time the arterial concentration of the tracer was monitored with repeated blood samples from the brachial artery into glass capillaries with pointed tips. A

known aliquot was then taken for determination of the radioactivity by B-scintillation counting. The autoradiographic processing of the brain was identical to the procedure for determination of the local CMRgl.

Calculations: The glucose utilization was calculated using the formula given by Sokoloff et al (1977). In all experiments, the standard values for the transport constants and the lumped constant were used. In the seizure group, where a slight change of the lumped constant would be expected to take place in certain structures, values calculated in the standard way and modified according to a previous description (Ingvar and Siesjö 1983, 1985) are presented (see discussion). The l-CBF was calculated according to the formula given by Sakurada et al (1978) using an iterative computer program.

The different areas in the brain were statistically regarded as independent entities. Significant differences from control were calculated using the Dunnett's multigroup comparisons test following analysis of variance (ANOVA).

RESULTS:

Table 1 depicts the physiological variables 10 min after the injection of isotope in the groups used for determination of the local cerebral glucose utilization, and immediately prior to the determination of the local cerebral blood flow. The values are similar to those of previous studies from the laboratory (Ingvar and Siesjö 1983, Ingvar et al 1984, Siesjö et al 1985), allowing correlation between the results during the seizure activity. Status epilepticus was accompanied by a slight systemic acidosis with pH values returning to normal over the 45 min long recovery period after termination of the seizure activity. It was possible to maintain the pCO_2 and the

pO_2 within the predetermined limits for all groups. All measurements of l-CBF were performed during a steady blood pressure, and there was no statistical difference between the pressures recorded in the different groups.

In table 2 the absolute values for the l-CMRgl are presented. The control values are in agreement with previous values presented from our laboratory (Ingvar and Siesjö 1983), using nitrous oxide/oxygen ventilated animals.

The values for the seizure group are presented as calculated with the standard lumped constant of 0.48 given by Sokoloff (Sokoloff et al 1977).The values obtained in the seizure group were very similar to the values previously reported for bicuculline-induced seizures (Ingvar and Siesjö 1983). The most marked increases were seen in the neocortex, the limbic areas, the hypothalamus and the substatia nigra, whereas e.g. the colliculi and the geniculate bodies showed no or only small increases.

In the immediate post-ictal period, the metabolic rate was depressed in the neocortical regions (range 46%-66% of control). This metabolic depression was less expressed in the neocortical regions in the 45 min recovery group (range 52-80% of control). In contrast to the cortical areas, the limbic areas did not show a marked depression of CMRgl. The regions with the highest metabolic rate during seizures had the most pronunced depression of metabolic rate in the early postictal period ($r=0.46$, $p<0.05$).

In table 3, the absolute values for the lCBF are given. After 20 min of seizure activity there was an increase in lCBF in the majority of structures. Thus, in the the neocortical regions the blood flow had increased to approximately 200% of control. In septal nucleus,

Table 1. Physiological parameters in the eight groups of the study. All values given are taken immediately before the determination of the cerebral blood flow and just prior to the termination of the isotope circulation period in the glucose utilization groups.

	Temp C	MABP mmHg	PO_2 mmHg	PCO_2 mmHg	pH	
			Local cerebral glucose utilization			
control n=5	36.6+0.2		147+7	98+4	36.2+0.7	7.37+0.03
5 min seizures n=3	37.4+0.3		110+3	>130	39.9+0.8	7.25+0.02
20 min seizures 5 min recovery n=4	36.6+0.1		116+3	108+6	35.6+1.1	7.36+0.01
20 min seizures 45 min recovery n=4	37.1+0.1		121+4	103+2	35.6+1.0	7.40+0.01
			Local cerebral blood flow			
control n=6		36.8+0.2	137+4	100+5	36.4+1.4	7.38+0.02
20′ seizures n=6		36.8+0.2	113+2	136+15	39.4+1.9	7.28+0.02
20′seizures 5′ recovery n=7		37.4+0.2	110+3	105+10	40.4+1.3	7.28+0.01
20 seizures 45′ recovery ·=7		36.9+0.2	121+2	102+5	37.3+0.9	7.35+0.01

ΔBP= Mean arterial blood pressure. Values are given as mean+SE

Table 2. Local cerebral metabolic rate for glucose in the rat brain during and following flurothyl induced status epilepticus.

	Control	Seizures 5′	Seizures 20′ 5′ recovery	Seizures 20′ 45′ recovery
Frontal Cortex	0.60+0.06	1.79+0.10*	0.39+0.04	0.48+0.03
Caudoputamen	0.62+0.03	1.40+0.08*	0.60+0.07	0.69+0.05
Globus Pallidus	0.28+0.02	2.38+0.11*	0.22+0.04	0.33+0.04
Substantia Nigra	0.38+0.02	1.63+0.08*	0.36+0.07	0.46+0.05
Red Nucleus	0.52+0.04	0.94+0.16*	0.47+0.07	0.53+0.04
Nc Accumbens	0.61+0.04	1.13+0.10*	0.36+0.02*	0.48+0.03
Cerebellum	0.26+0.03	0.72+0.14*	0.26+0.04	0.23+0.02
Sensorimotor Cortex	0.54+0.02	1.75+0.12*	0.35+0.03	0.39+0.04
Parietal Cortex	0.56+0.03	1.61+0.12*	0.32+0.04*	0.44+0.03
Thalamus	0.78+0.05	1.43+0.08*	0.50+0.04*	0.66+0.06
Visual Cortex	0.60+0.06	1.74+0.17*	0.35+0.04	0.38+0.04
Lateral Geniculate Body	0.64+0.06	0.98+0.08*	0.39+0.06*	0.51+0.05
Superior Colliculus	0.54+0.03	1.05+0.17*	0.52+0.06	0.63+0.06
Auditory Cortex	0.89+0.08	1.67+0.13*	0.41+0.05*	0.47+0.03*
Medial Geniculate body	0.79+0.07	1.21+0.09*	0.43+0.06*	0.55+0.04
Inferior Colliculus	0.84+0.11	0.69+0.52	1.06+0.18	1.06+0.08
Entorhinal Cortex	0.48+0.02	1.87+0.24*	0.41+0.04	0.38+0.02
Cingulate Cortex	0.72+0.05	1.57+0.96*	0.36+0.05*	0.52+0.04
Hippocampus	0.40+0.01	1.82+0.12*	0.54+0.09	0.46+0.04
Amygdala	0.45+0.02	1.35+0.07*	0.36+0.04	0.51+0.05
Septal Nucleus	0.30+0.03	1.90+0.17*	0.26+0.03	0.31+0.03
Habenula	0.52+0.05	1.06+0.18*	0.56+0.05	0.64+0.05
Hypothalamus	0.30+0.02	1.51+0.03*	0.27+0.02	0.38+0.03*

values are given in umol/g/min and represent Mean + SE
* denotes p<.01 from control

Table 3. Local cerebral blood flow in the rat brain during and following flurothy induced status epilepticus.

	Control	Seizures 20′	Seizures 20′ 5′ recovery	Seizures 20′ 45′ recovery
Frontal Cortex	2.16+0.28	3.36+0.33*	0.71+0.07*	0.91+0.13*
Caudoputamen	1.44+0.10	1.50+0.16	0.80+0.06*	0.69+0.05*
Globus Pallidus	0.87+0.10	2.96+0.15*	1.26+0.24	0.61+0.06
Substantia Nigra	1.32+0.13	4.65+0.31*	2.13+0.34	1.32+0.27
Red Nucleus	1.83+0.20	5.18+0.50*	3.19+0.47	1.61+0.15
Nc Accumbens	2.64+0.46	5.22+0.60*	2.46+0.62	1.10+0.13
Cerebellum	1.14+0.07	2.15+0.27	1.15+0.14	1.12+0.05
Sensorimotor Cortex	2.18+0.25	4.40+0.73*	0.72+0.04	1.04+0.14
Parietal Cortex	2.15+0.60	4.12+0.48*	0.67+0.06*	0.75+0.05*
Thalamus	2.32+0.33	5.33+1.97*	1.18+0.14	1.15+0.11
Visual Cortex	1.60+0.14	3.43+0.49*	0.60+0.07	0.68+0.06
Lateral Geniculate Body	1.77+0.17	4.80+0.85*	0.97+0.10	0.93+0.09
Superior Colliculus	1.80+0.21	6.42+0.47*	3.31+0.54	1.49+0.10
Auditory Cortex	2.81+0.30	4.44+0.52*	0.94+0.10*	1.35+0.16
Medial Geniculate body	3.65+0.95	5.79+0.78	1.55+0.14	1.26+0.09*
Inferior Colliculus	3.03+0.52		3.87+0.39	2.86+0.43
Entorhinal Cortex	1.45+0.13	3.09+0.27*	0.67+0.06*	0.45+0.02*
Cingulate Cortex	1.83+0.17	5.80+0.91*	0.98+0.10	1.13+0.20
Hippocampus	1.03+0.08	2.00+0.39*	0.60+0.04	0.44+0.03
Amygdala	1.28+0.13	3.61+0.28*	0.80+0.09	0.56+0.03*
Septal Nucleus	1.02+0.06	4.07+0.33*	2.79+0.67	0.76+0.05
Habenula	1.84+0.21	4.83+0.66*	1.61+0.12	1.25+0.10
Hypothalamus	1.25+0.15	4.38+0.25*	2.64+0.45*	1.05+0.07

values are given in ml/g/min and represent Mean + SE
* denotes p<.01 from control

hypothalamus, amygdala, globus pallidus, substantia nigra, red nucleus, and superior colliculus the lCBF surpassed 300% of control. No increase was noted in the caudoputamen at 20 min of seizure activity. No value is reported for the inferior collicuclus in the seizure group, as the increase in CBF in this region was out of the dynamic range for the lCBF method as applied in this paper (square wave infusion of isotope over 20 s). The pattern of flow is similar to the pattern described during seizures induced by either bicuculline or pentylenetetrazol (Ingvar and Siesjö 1983, Ingvar et al 1984).

In the postictal period, the blood flow was markedly reduced in most areas when compared to the control situation. The lCBF in the neocortical regions had decreased to values between 30 and 50 % of control in the 5 min recovery group. However, no decreases and, in some cases, even increases were seen in mesencephalic structures like the substantia nigra, the red nucleus, the colliculi as well as in the hypothalamus, the globus pallidus and the septal nucleus. The pattern of lCBF in the 5 min recovery group was neither correlated to the control group nor to the pattern of l-CBF during seizures. Thus, in contrast to the CMRgl, the severity of the postictal depression of blood flow did not correlate to the increases during the ictal period.

Fortyfive min after the arrest of the 20 min period of status epilepticus the neocortical regions still had a depressed blood flow to between 30 and 60 % of control. In this group, no region had an increased flow compared to the control situation. The flow pattern was returning towards normal as indicated by a linear correlation between the control flow values and the CBF for this group ($r=.57$, $p<.01$).

In figure 1, values for both lCMRgl and lCBF are plotted for six different structures. In this figure, the values for the metabolic rate during seizures have been corrected for the change in the lumped constant during seizures (see discussion). To the left, it is shown that neocortical regions had a marked increase of the metabolic rate during seizures followed by a marked depression in the post-ictal period. Local CBF increased almost to the same extent, but the depression following arrest of the seizure activity seemed even more pronounced than for lCMRgl. In the hippocampus, the marked increase during seizures was not followed by depression in the metabolic rate during the post-ictal phase. The superior colliculus and the red nucleus demonstrate how regions with only minor increases in the metabolic rate but large increases in blood flow during seizures (cf Ingvar and Siesjö 1983) do not have a depressed metabolic rate in the postictal period.

Coupling between metabolic rate and blood flow during and following seizures: In the seizure group, no coupling between metabolic rate and blood flow was detectible. This is in agreement with the previous report where blood flow and metabolic rate were measured during bicuculline-induced seizures (Ingvar and Siesjö 1983). The distributions of both metabolic rate and blood flow were very similar in the two models as illustrated in figure 2. Here, the ratio between CMRgl and CBF for each structure obtained during seizures with either bicuculline or flurothyl are plotted. A significant correlation was obtained ($r=0.88$, $p<0.01$).

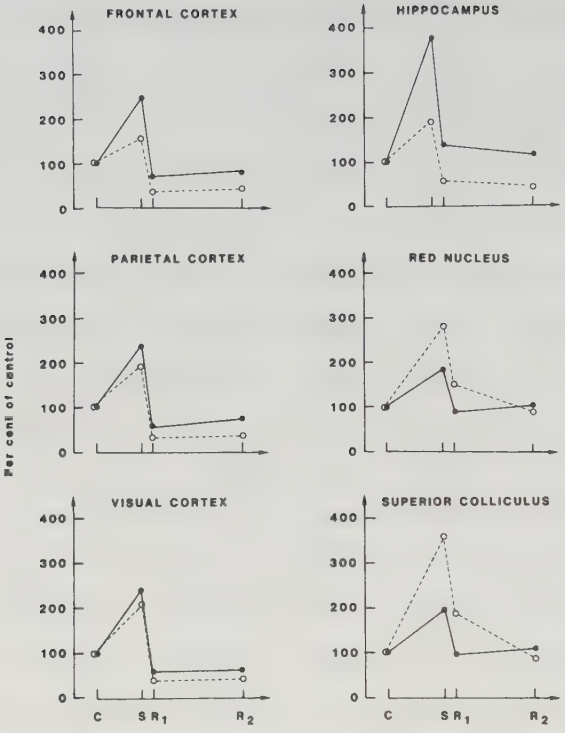

Figure 1. lCMRgl (solid lines) and lCBF (hathched lines) in six different structures in the rat brain. Values are given as per cent of control for control animals (C), animals with seizures (S) and for early (R1) and late (R2) recovery groups. Values for metabolic rate during seizures are adjusted for the changes in the tissue to plasma glucose concentration ratios (se discussion). To the left is illutrated the marked increases in the metabolic rate and blood flow during seizures followed by a marked longstanding depression of the metabolic rate in the neocortical areas. In contrast there was no metabolic depression in the hippocampus following seizures. The two lower right graphs depict regions with less marked increase in the metabolic rate, but with marked flow increases during seizures. In these regions both blood flow and metabolic rate rapidly normalize in the postictal period.

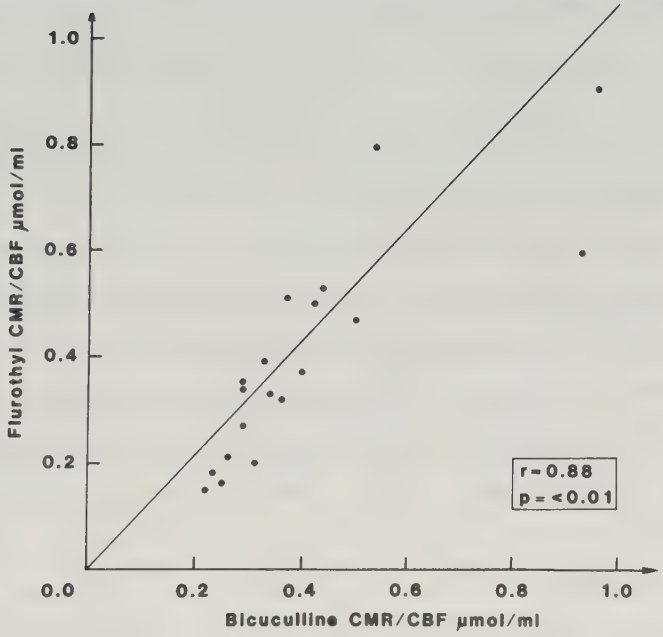

Figure 2. A plot of the ratio between the metabolic rate of glucose and blood flow for 19 cerebral structures. The ordinate represents ratios calculated from the previously reported values for status epilepticus induced with bicuculline (Ingvar and Siesjö 1983), and the abscissa denotes the present results in the seizure groups. The abolishment of the coupling between the cerebral blood flow and metabolism on the regional level during bicuculline seizures is thus also present during seizures induced with flurothyl.

The coupling between CMRgl and lCBF had not returned 5 min after arrest of the seizure activity, whereas after 45 min a correlation between the two variables ($r=0.73$, $p<0.01$) indicated a return of the normal coupling.

DISCUSSION:

Before turning to the main results, a comment on the use of the deoxyglucose method is warranted. During seizures, the increased metabolic rate in some regions will induce a change in the tissue to plasma glucose concentration ratio (Chapman et al 1977, Ingvar and Siesjö 1983, Folbergrova et al 1985). This will in turn induce a regional increase in the value of the lumped constant (Pardridge et al 1982 a,b, Gjedde 1982). Thus, values for lCMRgl calculated by use of the normal lumped constant of 0.48 will yield an overestimation of the metabolic rate in the regions where large increases in metabolic rate are present. The changes are limited if the tissue glucose concentration is maintained above control and predictable if the changes in the glucose concentration ratios are known. The values presented in table 2 are all given as calculated with a lumped constant of 0.48. In figure 1, however, the values are modified according to the changes in the glucose concentration ratios during flurothyl seizures (Folbergrova et al 1985, cf Pardridge et al 1982 a,b, Ingvar and Siesjö 1983). The changes in the calculated values for metabolic rate by this modification are relatively small during seizures in ventilated rats with normal or high plasma glucose concentrations. The modification should be done only in regions with a

very marked increase of the metabolic rate. We wish to emphasize that such changes in the lumped constant do not influence the conclusions drawn in this paper.

Local CBF and CMRgl in the postictal period: Previous reports on postictal CBF have demonstrated a depression of CBF after generalized seizures in patients (Grant et al 1947, Kety et al 1948, Meyer et al 1966, Magistretti et al 1983). The data presented here for the group measured 5 min after termination of the 20 min period of SE agree with the previously presented data. However, by means of the regionality of the methods used, we could show that the CBF depression is inhomogenous and most pronounced in the neocortical regions. It is unclear why some regions (e.g the septal nucleus) have a persistent increase in the cerebral blood flow 5 min after the termination of the status epilepticus. The EEG did not reveal any ongoing ictal activity 5 min after termination of exposure to the flurothyl. The rapid fall in CBF and CMRgl following the arrest of seizures underlines how quickly the brain can alter its physiological state, and thereby emphasizes the care one must take e.g. when studying seizures with the deoxyglucose techniques (see below).

Somewhat surprisingly, lCMRgl and lCBF were still severely suppressed below control 45 min after the seizure arrest. The reduction of CBF following 20 min status epilepticus, is of similar magnitude as that observed after a period of insulin-induced hypoglycemia or following ischemia (Abdul Rahman and Siesjö 1981, Kågström et al 1983). The reduced perfusion after seizures is probably irrelevant regarding the development of neuronal damage following seizures. It is known that seizure periods lasting only 20 min do not

result in any permanent brain damage (Nevander et al 1985). Also, the decrease in blood flow postictally in the cerebral cortex does not seem to decrease the substrate availability in the cortex. Folbergrova et al (1985) showed that the tissue to plasma glucose concentration ratio was increased in the period following status epilepticus.

Local CMRgl and CBF during induced status epilepticus:

Several groups have used the deoxyglucose technique during chemically induced seizures and found regional differences in the metabolic activation for different convulsants (Cudennec et al 1985, Pazdernik et al 1985). However, as the seizures were not maintained for the duration of the CMRgl measurement during the testing of the different drugs, it is not possible to draw conclusions on the similarities/dissimilarites of the elicited seizure patterns. Even though bicuculline and allylglycine both elicit seizures via GABA inhibition (GABA receptor blocking and GAD inhibition, respectively (Woodbury 1983)), Evans and Meldrum (1984) concluded that the metabolic activation patterns were not the same for these two drugs. However, the animals subjected to bicuculline seizures were fasted and had plasma glucose levels below 5 μmol/ml. Such bicuculline-induced seizures lead to a progressive fall in plasma glucose and to a decrease in seizure intensity (Blennow et al 1978, 1979). The inability to meet the steady state criteria for the deoxyglucose method (Sokoloff et al 1977) could explain why these results are at variance with the previously reported CMRgl values for bicuculline seizures (Ingvar and Siesjö 1983). The animals with seizures induced with allylglycine had 8 μmol/ml in plasma glucose. Since the glucose

delivery then is not limiting the glucose use, pronounced increases in the metabolic rate were reported for allylglycine (Evans and Meldrum 1984).

Flurothyl, bicuculline and pentylenetetrazol have been shown to induce similar acute histopathological alterations and similar changes in the labile energy metabolites (Chapman et al 1977, Folbergrova et al 1981, Söderfeldt 1981, Howse 1983, Ingvar et al 1984, Folbergrova et al 1985). We have also conluded that the changes in local cerebral blood flow were the same in seizures induced by either bicuculline or pentylenetetrazol (Ingvar and Siesjö 1983, Ingvar et al 1984). Flurothyl has the same distribution of flow and metabolic rate during seizures as bicuculline ($r=0.90$, $p<0.001$ and $r=0.72$ $p<0.001$, respectively) (Ingvar and Siesjö 1983) and pentylenetetrazol (preliminary observation). The regional coupling between the metabolic rate and blood flow during flurothyl seizures is severely deranged. The very good correlation obtained when plotting l-CMRgl/l-CBF for the different areas (Figure 2) indicate that this regional uncoupling between the two variables follow the same pattern in flurothyl- and bicuculline-induced seizures. We conclude that the seizures induced by either flurothyl, bicuculline or pentylenetetrazol is identical in spite of their different mechanisms of action (Woodbury 1983), provided the physiological parameters are standardized and the EEG patterns elicited are similar.

pHe and the regulation of the cerebral blood flow: The factors regulating the coupling of CBF and cerebral metabolism are not completely elucidated. Among those suggested, extracellular pH (pHe) has been suggested to be the most important factor in the local regulation of the cerebrovascular resistance (Lassen 1968, Howse et al

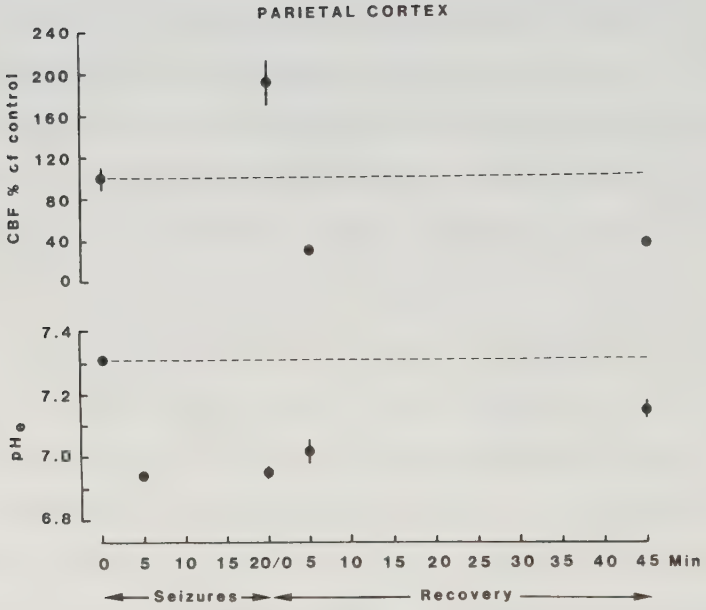

Figure 3. CBF calculated as per cent of control in the parietal cortex during and following flurothyl-induced seizures. For comparison, data for the extracellular pH during the same time period, measured by use of the same animal preparation (Siesjö et al 1985), are displayed in the lower section of the figure. Note that in spite of the marked reduction of the blood flow in the period following seizures, extracellular pH does not markedly change.

1974). However, later experiments have revealed a dissociation between the CBF and extracellular pH during the initial phase of seizure activity (Astrup et al 1976, Nilsson et al 1976). In spite of this apparent dissociation, it was recently proposed that the pHe is responsible for the maintenance of an increased blood flow during prolonged seizure activity (Leniger-Follert 1984). The data presented in figure 3 show the changes in local cerebral blood flow over time as compared with the changes in extracellular pH for the same measurement period (pH data from Siesjö et al 1985). The l-CBF in the parietal cortex decreases to subnormal values upon the spontaneous arrest of the seizure activity, but the extracellular pH in the same region remains acidotic. We thus conclude that the CBF is not coupled to the cerebral metabolic rate via changes in extracellular pH. Alternative mechanisms, yet undefined, are responsible for the local regulation of the cerebral blood flow in this situation.

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Extra- and Intracellular pH in the Brain During Seizures and in the Recovery Period Following the Arrest of Seizure Activity

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Summary: The objective of the study was to estimate changes in extracellular pH (pH_e) and intracellular pH (pH_i) during seizures and in the recovery period following the arrest of seizure activity. Seizures of 5- and 20-min duration were induced in rats by fluorothyl added to the insufflated gas mixture, and recovery for 5, 15, and 45 min was instituted by withdrawal of the fluorothyl supply following 20 min of continuous seizures. Changes in pH_e were measured by double-barreled, liquid ion-exchange pH microelectrodes, and in pH_i by the CO_2 method, following estimation of tissue P_{CO_2} and extracellular fluid (ECF) volume. The animals were either normoxic or rendered moderately hypoxic (arterial P_{O_2} 40–50 mm Hg). Upon induction of seizures in normoxic animals, pH_e decreased by a mean of 0.36 unit, the values being identical at 5 and 20 min. In moderate hypoxia, seizures sustained for 20 min were accompanied by a further fall in pH_e (mean decrease 0.51 unit). The changes in pH_e seemed mainly to reflect the nonionic diffusion of lactic acid from cells to the ECF (tissue lactate levels ~10 and 15 μmol g^{-1} during seizures in normoxic and hypoxic animals, respectively). However, the gradual fall in pH_e attributable to lactic acid production was preceded by rapid acidification, sometimes exceeding the steady-state values subsequently attained. This acidification was interpreted to reflect spreading depression and fast transcellular Na^+/H^+ exchange. Following cessation of seizure discharge, pH_e normalized at a surprisingly slow rate, with some acidosis persisting even after 45 min. The difference between cerebrovenous and arterial P_{CO_2} was reduced during seizures and increased in the recovery period, probably reflecting alterations in the blood flow/metabolic rate coupling. Impedance changes were slight, indicating only minor changes in ECF volume. Changes in pH_i after 5 min of seizures ranged from 0.20 (normoxic animals) to 0.32 (hypoxic animals) unit, the pH_i values after 20 min being 0.07–0.08 unit higher. The results suggest the regulation of pH_i during ongoing seizures. Upon arrest of seizure activity, pH_i rapidly increased to normal and subsequently to supranormal values. Postepileptic intracellular alkalosis occurred at a time when pH_e was still reduced and in spite of the fact that tissue lactate values had not normalized. It is concluded that the rapid normalization of pH_i and overt alkalosis were caused by the simultaneously occurring oxidation of lactate, with the removal of a stoichiometrical amount of H^+, and the extrusion of H^+ from cells, possibly via a Na^+/H^+ exchanger, the latter probably delaying normalization of pH_e. **Key Words:** Cerebral extracellular pH—Cerebral intracellular pH—Lactic acidosis—Seizures.

Seizures induced in normo- or hyperglycemic animals invariably lead to tissue lactic acidosis. During sustained seizures, the tissue lactate content increases to, and remains at, values of 8–10 μmol g^{-1} (Duffy et al., 1975; Chapman et al., 1977; Folbergrová et al., 1981). If cerebral oxygenation is curtailed by induced arterial hypoxia or hypotension, tissue lactate values may reach or exceed 20 μmol g^{-1} (Blennow et al., 1977). On the other hand, if animals become hypoglycemic during seizures, the increase in lactic acid content is small or absent (Blennow et al., 1979; see also Folbergrová et al., 1981).

Although it seems clear that the production of lactic acid during seizures must reduce the extra- and intracellular pH (pH_e and pH_i, respectively), little information exists on the degrees of acidosis attained during sustained seizures. Several studies have been devoted to measurements of pH_e with glass electrodes during short-lasting seizures (Jasper and Erickson, 1941; Wang and Sonnenschein, 1955; Caspers and Speckmann, 1972; Howse et al., 1974; Astrup et al., 1978; Heuser, 1978, 1981). Some of the authors used either rela-

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Abbreviations used: ECF, extracellular fluid; pH_e, extracellular pH; pH_i, intracellular pH; P_tCO_2, tissue CO_2 tension; TCO_2, total tissue CO_2 content.

tively bulky microelectrodes, which may have caused some tissue damage, or surface electrodes resting on the pia-arachnoid membrane, the pH of which bears an uncertain relationship to the pH of the extracellular fluid (ECF) proper. In fact, one group reported that such electrodes recorded a decrease in pH during seizures only if the P_{CO_2} increased (Howse et al., 1974). In none of the studies cited were the recorded pH shifts correlated with tissue lactic acid values.

Even less information is at hand regarding changes in pH_i. Howse et al. (1974) induced seizures of 40- to 60-s duration and measured tissue CO_2 content after 10 or 30 s of seizure activity as well as 0.5, 5, and 15 min following the arrest of seizures. Their calculations suggest that pH_i fell by ~0.15 unit for an increase in tissue lactate content of 5.5 μmol g^{-1}. However, the authors made no attempt to correct for changes in ECF HCO_3^- content. Two subsequent studies provide information on changes in pH_i as calculated from the creatine kinase equilibrium (Siesjö et al., 1972). In one of these, a single electroshock and 1 min of pentylenetetrazole-induced seizures reduced pH_i, so calculated, by 0.09 and 0.16 unit, respectively (Duffy et al., 1975). In the other, in which bicuculline-induced seizures were sustained for periods varying between 10 s and 20 min, the change in pH approximated 0.2 unit (Chapman et al., 1977; Folbergrová et al., 1985).

The present study was undertaken to provide quantitative data on changes in pH_e and pH_i during seizures sustained for 5 or 20 min, and to assess pH_e and pH_i during the recovery following the arrest of seizure activity. We induced seizures by the convulsant gas fluorothyl, which was added to the inspired gas mixture and retained in a rebreathing system (Nevander et al., 1984; Folbergrová et al., 1985). The method allowed seizures to be arrested by discontinuation of the fluorothyl supply, obviating the need to use anticonvulsant drugs. Changes in pH_i were derived by the CO_2 method. Tissue CO_2 tensions (P_tCO_2) were evaluated from measured values of arterial and cerebrovenous P_{CO_2}. To estimate changes in pH_e, liquid ion-exchange microelectrodes were inserted into the cerebral cortex, and changes in ECF volume were assessed by impedance measurements.

MATERIALS AND METHODS

Two recent articles describe the type of animals used, most of the operative procedures, and the technique for inducing, maintaining, and arresting seizure activity (Nevander et al., 1984; Folbergrová et al., 1985). In summary, fed male rats of S.P.F. Wistar strain were anesthetized with 3% halothane–70% N_2O, maintained during operation on 1% halothane–70% N_2O, and studied while ventilated on 70% N_2O–30% O_2. Immobilization was achieved with suxamethonium, and the animals were artificially ventilated. Seizures were induced by adding 80 μl fluorothyl to the inspired gas mixture, sustained by retaining the convulsant in a rebreathing system, and arrested by discontinuing the fluorothyl supply. Femoral arterial and venous catheters were inserted for the control of blood pressure, sampling of blood for measurements of pH and blood gases, and infusions. Body temperature was maintained close to 37°C. EEG was recorded by bipolar leads from screws inserted in the calvarium of the frontoparietal region. In the following, we describe additional techniques in more detail.

Experimental series

Four experimental series were studied. One was used to measure the total CO_2 content (T_{CO_2}) of frontoparietal neocortical samples, taken from the hemisphere opposite to that used for the analysis of labile metabolites. An additional series was used to assess arterial and cerebrovenous P_{CO_2} during and following seizures. In these animals, a burr hole was placed over the caudal part of the superior sagittal sinus to allow sampling of cerebrovenous blood (see Pontén and Siesjö, 1966; MacMillan and Siesjö, 1973). Yet another series was used for measuring pH_e with double-barreled pH microelectrodes. To achieve this, a craniotomy (~3 × 3 mm) was placed over the frontoparietal cortex, the dura was carefully incised, and the cortical surface was kept moist with artificial CSF added to a small plastic funnel attached to the bone surrounding the craniotomy. The CSF was prewarmed to 37°C and equilibrated with ~5% CO_2. A common ground lead was provided by placing a macro reference electrode (0.16 *M* KCl in agar) in a separate burr hole in a more frontal position. In six of the animals, the tissue was frozen in situ after removal of the microelectrode, either under control conditions (n = 1) or after 20 min of seizure activity (n = 5), to allow measurements of tissue lactate content. In two additional animals, tissue was frozen in situ after 1 min of seizure activity. Samples (~10 mg) from the electrode side were dissected at −22°C from the surroundings of the electrode tip. The last series was used to monitor tissue impedance changes during and following seizures. For these measurements, a craniotomy was placed as in the experiments designed for pH_e measurements.

Measurements of pH_e and pH_i

pH_e. Changes in pH_e were assessed by double-barreled liquid ion-exchange electrodes with tip diameters of 1–3 μm. The electrodes were constructed as described in two recent communications (Kraig et al., 1983; Mutch and Hansen, 1984). Following silanization, the tip of the pH barrel was filled with the pH "cocktail" developed by Ammann et al. (1981), and the barrel was back-filled with a phosphate buffer (pH 6.5). The reference barrel contained 0.16 *M* KCl. The tip of the electrode was then beveled at an angle of 90°. The barrels were connected via Ag-AgCl electrodes to a high-impedance differential amplifier.

The electrodes were calibrated at 37°C in a series of phosphate buffers (pH 6.0–7.8) to check for sensitivity to changes in pH and for absence of drift. In some early

experiments, this in vitro calibration was the only one performed. It soon became clear, though, that although this procedure must give correct values for changes in pH (ΔpH), it gave too low absolute pH values. Thus, when the double-barreled electrode was withdrawn from the cortex and the funnel overlying the tissue surface was repeatedly filled with fresh buffer solution, the calibration curve shifted in parallel fashion along the pH axis, demonstrating that the true pH_e was close to 7.3 (see below).

As a test of electrode performance, we measured pH_e during short-lasting (5–10 min) hypercapnia, P_aCO_2 being increased to 70–80 mm Hg. About 15–20 min later, when pH_e had stabilized at prehypercapnic values, seizures were induced by the administration of fluorothyl. In some animals, the experiments were terminated after 20 min of seizure activity to allow the freezing of tissue in situ and estimation of tissue lactate content. However, in four normoxic and four hypoxic animals, the pH_e was continuously monitored during 20 min of seizures and for 40–45 min following the discontinuation of seizure activity. In one of these, seizures were arrested by the intravenous administration of 15 mg kg^{-1} thiopental.

pH_i. Changes in pH_i were derived by the CO_2 method. According to this method, the intracellular HCO_3^- concentration ($[HCO_3^-]_i$) and pH_i are calculated from the following equations (Siesjö et al., 1972):

$$[HCO_3^-]_i = \frac{Tco_2 - P_tCO_2 \cdot 0.0292 - V_{ECF}\,[HCO_3^-]_{ECF} - V_{Bl}\,[HCO_3^-]_{Bl}}{V_i} \quad (1)$$

$$pH_i = 6.12 + \log \frac{[HCO_3^-]_i}{P_tCO_2 \cdot 0.0314} \quad (2)$$

In these equations, Tco_2 is the total CO_2 content (μmol g^{-1}); P_tCO_2 is the tissue CO_2 tension (mm Hg); 0.0292 is the solubility of CO_2 in brain tissue (μmol g^{-1} mm Hg^{-1}); V_{ECF}, V_{Bl}, and V_i are the volumes occupied by ECF, blood, and intracellular fluid, respectively; 6.12 is the pK_1' of H_2CO_3; and 0.0314 is the solubility of CO_2 in intracellular fluid (μmol g^{-1} mm Hg^{-1}).

For each experimental situation (control; 5 and 20 min of seizure activity; 5, 15, and 45 min of recovery), P_tCO_2 was calculated from the following equation (Pontén and Siesjö, 1966):

$$P_tCO_2 = \frac{P_aCO_2 + P_vCO_2}{2} + 1 \quad (3)$$

As mentioned above, P_aCO_2 and P_vCO_2 were measured in a separate series of experiments.

We derived the extracellular HCO_3^- concentration ($[HCO_3^-]_e$) from the Henderson-Hasselbalch equation, using the values for pH_e and P_tCO_2 and a pK_1' of 6.12. V_{ECF} was assumed to be 18% (0.18) of tissue weight for the control situation, giving a V_i of 0.58 (see Siesjö et al., 1972). Based on changes in tissue impedance (see below), we corrected the ECF volume and adjusted the intracellular volume accordingly.

Measurements of tissue impedance

Cerebral cortical impedance was measured by the four-electrode technique of Ranck (1966), as described in detail in a previous publication (Pelligrino et al., 1981). The tips of the electrodes were inserted into the exposed cortical surface to a depth of ~500–700 μm. Changes in ECF volume from an assumed control value of 18% were calculated by the use of the Maxwell and Raleigh equations (see Pelligrino et al., 1981).

RESULTS

We describe in turn seizure-induced changes in P_tCO_2, in pH_e, and in pH_i, before relating changes in pH to cerebral metabolic events, notably the production of lactic acid.

Blood and tissue P_{CO_2}

In the majority of animals, arterial P_{CO_2} was in the range of 35–40 mm Hg. Figure 1 shows alterations in the P_{CO_2} difference between cerebrovenous and arterial blood during seizures of 5- and 20-min duration and in the recovery period, and gives the ΔP_{CO_2} values that, in subsequent experiments, were added to the measured P_aCO_2 to give P_tCO_2. The control arteriovenous P_{CO_2} difference was 8.2 ± 0.3 mm Hg (mean ± SEM). This decreased during seizures and rose during recovery. Probably, these changes occurred because the blood flow increased proportionally more than the metabolic rate during seizures, and decreased in excess of the metabolic rate during recovery. As a result, the ΔP_{CO_2} derived (lower panel) was lower during seizures than in the recovery period.

pH_e—normoxia

When calibrated in buffers at 37°C, the electrodes gave a linear response, with a slope of 57.5 ± 0.9 mV per unit change in pH. Pre- and postexperiment in vitro calibrations showed shifts that usually did not exceed 3 mV. In all instances, the slope of the curves remained unchanged, attesting to the accuracy of the pH changes recorded.

As Figure 2 shows, addition of CO_2 to the insufflated gas mixture gave rise to an acid shift in pH_e, recorded as an upward deflection of the tracing, which normalized within a few minutes following discontinuation of the CO_2 supply. To assess the rate of recovery after hypercapnia of longer duration, a few animals were allowed to inhale CO_2 for 20 min. As seen in Fig. 2, even in these cases recovery was complete within 10 min. In the nine animals studied, pH_e changed by 0.23 ± 0.02 unit for a mean P_{CO_2} change of 41 mm Hg. In these exper-

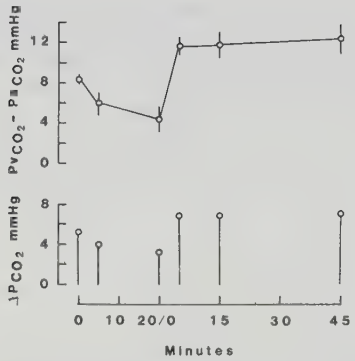

FIG. 1. Cerebrovenous–arterial (P_vCO_2–P_aCO_2) and tissue–arterial (ΔPCO_2) differences in CO_2 tension during 20 min of fluorothyl-induced seizures and following arrest of seizure activity. Values are means ± SEM (P_vCO_2–P_aCO_2) or means (ΔPCO_2); n = 6.

iments, no attempt was made to achieve a square-wave change in PCO_2. Thus, failure to reach a plateau value for pH_e could reflect, at least in part, a gradual increase in PCO_2.

Figure 3 gives two representative records from animals with seizures of 20-min duration. As observed, the epileptic discharge was picked up by the microelectrode. In all eight experiments, the reduction of pH_e showed a biphasic pattern, the first change appearing as a fast acidification followed by partial and transient recovery. In most experiments, pH_e then fell to reach a stable value within the first 5 min (upper tracing). In three experiments, though, the initial acidification exceeded the stable values subsequently reached (lower panel). We interpreted this initial, fast reduction in pH_e as reflecting an initial spreading depression (see Discussion).

The ΔmV values for the 5- and 20-min periods were read off the records and converted to ΔpH_e values by the use of the electrode calibration curve for each experiment. Table 1 gives the physiological variables, the mean ΔpH values, and the tissue lactate concentrations measured after 20 min of seizures in five of the nine experiments. The seizure animals were normothermic and all had blood pressures above 110 mm Hg. Arterial PCO_2 rose somewhat during seizures; however, owing to the change in ΔPCO_2 (see above), calculated P_tCO_2 values during seizures were below 45 mm Hg. Arterial pH fell somewhat during seizures. As observed, ΔpH_e showed little variation between animals, and the values were identical for the 5- and 20-min periods. On the side contralateral to the craniotomy (Contra), the tissue lactate content was similar to that previously measured in animals with an intact skull (Folbergrová et al., 1985). On the ipsilateral side (Ipsi), the values were 1 μmol g^{-1} higher, suggesting an influence of the craniotomy and electrode insertion. However, in the last two animals, the differences were only 0.31 and 0.11 μmol g^{-1}. In these, pH_e changes were similar to those measured in the remainder of the animals. We conclude, therefore, that tissue trauma did not contribute to the acid shift of pH_e.

One experiment allowed a comparison between

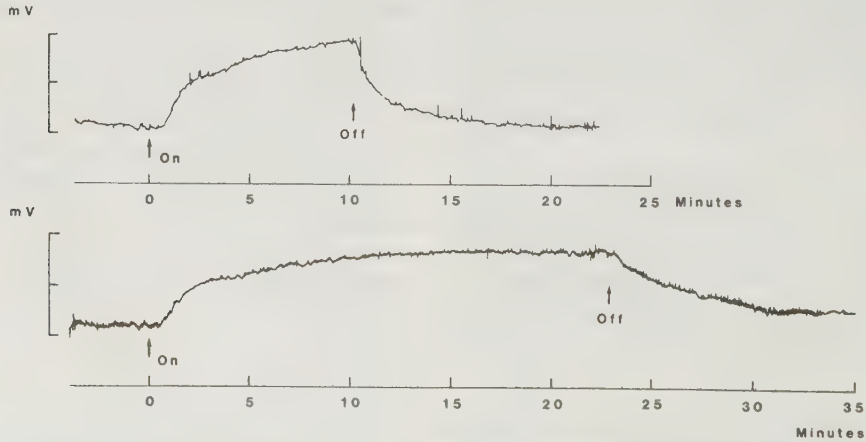

FIG. 2. Representative records of extracellular pH (pH_e) changes during hypercapnia of 10- or 20-min duration. Note rapid normalization of pH_e upon termination of hypercapnia. Scales on the left give electrode output in mV (the distance between two bars is 10 mV or ~0.17 pH unit).

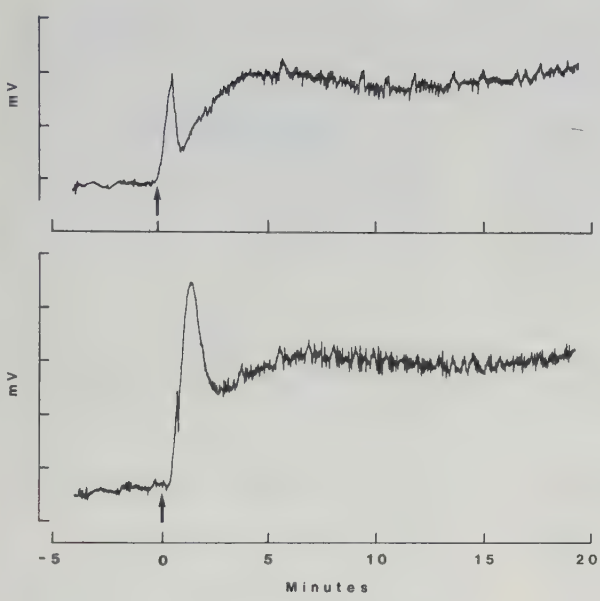

FIG. 3. Basic patterns of extracellular pH changes recorded during seizures of 20-min duration. In most animals, there was a rapid acidosis with partial recovery, and attainment of stable acidosis after 5 min **(top).** In some animals, the initial acidification exceeded that subsequently attained **(bottom).** Scales on the left give electrode output in mV (the distance between two bars is 10 mV or ~0.17 pH unit).

pH changes occurring during a CO_2 transient (ΔP_{CO_2} 37 mm Hg), an initial spreading depression, sustained seizure discharge, and final ischemia (Fig. 4). The pH changes were 0.22, 0.38, 0.32, and 0.70 unit, respectively.

The question arose of whether the initial pH shift ("spreading depression") was due to lactic acid production or to ionic shifts between intra- and extracellular fluids. In two animals in which tissue was frozen after 1 min of seizures, lactic acid values were 6.08 and 6.36 μmol g^{-1}, making it highly unlikely that lactic acid production was the cause of the marked acidosis (see Discussion).

As Fig. 5 shows, the recovery of pH_e following the arrest of seizure activity was surprisingly slow. Thus, relatively marked acidosis persisted after 15 min, and even after 45 min, pH_e was slightly lower than control. We note that this was in contrast to hypercapnia (see Fig. 2). Recovery was slow whether the seizures stopped spontaneously following withdrawal of the fluorothyl supply (upper record) or were arrested by thiopenthal (lower record). In the four animals studied, the persisting pH change after 5, 15, and 45 min of recovery was 0.29, 0.22 and 0.16 unit, respectively.

In situ calibration of the microelectrode was per-

TABLE 1. *Physiological parameters, cerebral cortical ΔpH_e values, and tissue lactate contents during fluorothyl-induced seizures*

Group (n = 9)	Temp. (°C)	MABP (mm Hg)	P_aCO_2 (mm Hg)	pH	ΔpH_e	Lactate (μmol g^{-1}) Ipsi	Lactate (μmol g^{-1}) Contra
Control	36.6 ± 0.1	154 ± 6	35.5 ± 1.1	7.34 ± 0.03	—	(1.64)	(1.51)
Seizures							
5 min	37.0 ± 0.0	123 ± 4	39.0 ± 1.8	7.27 ± 0.02	0.37 ± 0.01	—	—
20 min	37.1 ± 0.0	120 ± 3	39.5 ± 1.5	7.23 ± 0.02	0.36 ± 0.02	10.24 ± 0.28	9.29 ± 0.11

Values are means ± SEM. Lactate values were measured in samples from the ipsilateral (ipsi) and contralateral (contra) hemispheres, with respect to the site of the pH electrode (n = 6). Only one control animal was studied. pH_e, extracellular pH.

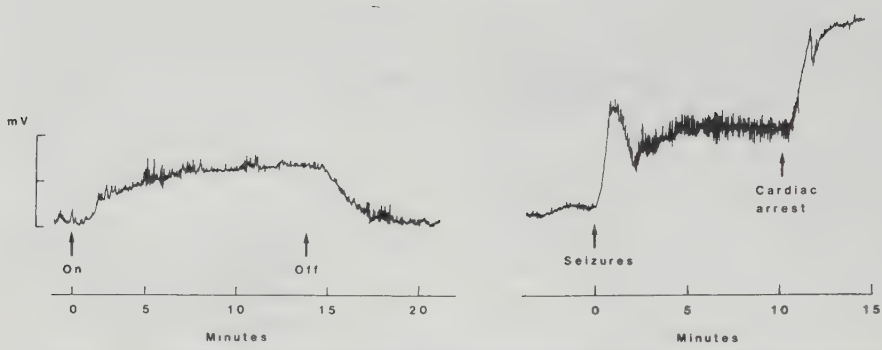

FIG. 4. Comparison of extracellular pH recordings during initial hypercapnia (**left;** ΔPco_2 = 31 mm Hg), during seizures with initial rapid acidification and subsequent stabilization, and during terminal anoxia (**right**). The pH changes for the four consecutive events were 0.22, 0.38, 0.82, and 0.70 unit. Scale on the left gives electrode output in mV (the distance between two bars is 10 mV or ~0.17 pH unit).

formed only in the last three normoxic experiments. However, in the subsequent hypoxic series, such calibrations were done. From the seven experiments available, the preepileptic pH_e was 7.31 ± 0.02. Since this value is similar to that reported by others (Kraig et al., 1983; Mutch and Hansen, 1984), we assumed that the preseizure pH_e was 7.31 in each experiment. It was then possible to calculate the HCO_3^- concentration from the Henderson-Hasselbalch equation. The numbers demonstrate that $[HCO_3^-]_e$ should have been reduced from 21 to 8–9 μmol ml^{-1}, i.e., by 12–13 μmol ml^{-1}, during seizures, and that recovery values at 5, 15, and 45 min were 11, 12, and 15 μmol ml^{-1}, respectively. These values were used to calculate pH_i, as described below.

pH_e—hypoxia

In the seven animals rendered moderately hypoxic (P_aO_2 40–50 mm Hg), other physiological variables were similar to those recorded in the normoxic animals (Folbergrová et al., 1985). After 5 min of hypoxic seizures, the mean ΔpH_e was 0.33 ± 0.02 unit, i.e., similar to the change in normoxic animals. After 5 min of reduced Po_2, ΔpH was 0.35 ± 0.03 unit, and after 20 min, ΔpH increased to 0.51 ± 0.04 unit. Thus, the exaggerated lactic acidosis was accompanied by a further fall in pH_e.

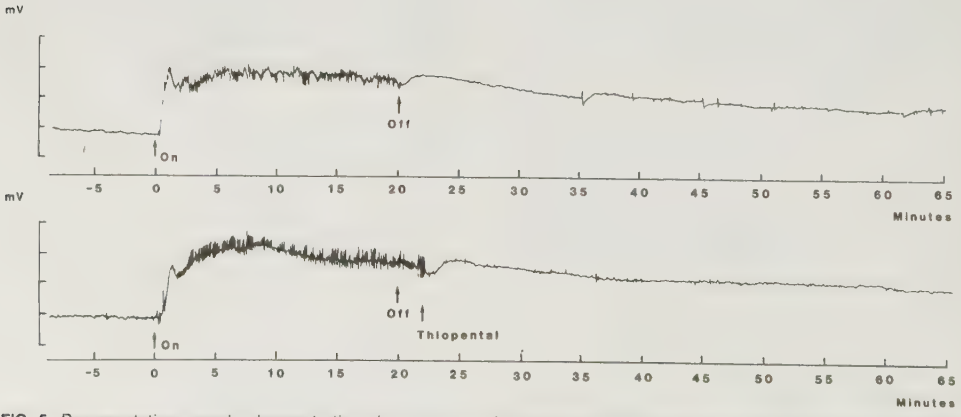

FIG. 5. Representative records, demonstrating slow recovery of extracellular pH following spontaneous **(top)** or thiopental-induced **(bottom)** arrest of seizure activity. Compare fast recovery following hypercapnia (Fig. 2). Scales on the left give electrode output in mV (the distance between two bars is 10 mV or ~0.17 pH unit).

Also, in this group, the recovery of pH was slow following the cessation of seizure discharge. After 5, 15, and 45 min, the persisting ΔpH was 0.30 ± 0.05, 0.17 ± 0.07, and 0.00 ± 0.07 unit, respectively. Although the last value suggests full normalization after 45 min, the variability was pronounced. The corresponding HCO_3^- concentrations, calculated from the pH and P_{CO_2}, were 11, 15, and 23 μmol ml^{-1}, respectively. These values were used to calculate changes in pH_i.

pH_i

Given the T_{CO_2} and the extracellular HCO_3^- concentration, it is possible to calculate the intracellular HCO_3^- concentration and pH_i, provided that the ECF volume is known. Changes in ECF volume were assessed by impedance measurements. The recording shown in Fig. 6 gives the impedance change during seizure discharge and that recorded during terminal anoxia. Seizures caused a surprisingly small change in impedance. In the example given, there was an unusually large, transient increase in impedance, which then almost normalized during ongoing seizures. In other experiments, the initial change was smaller and the sustained change somewhat larger. Generally, though, the changes were moderate. In seven experiments, the impedance change after 5 and 20 min of seizures was only 5% of control. As calculated by the Raleigh or Maxwell equations (Pelligrino et al., 1981), V_{ECF} decreased from an assumed normal value of 18% to 17.0 ± 0.03 and 16.7 ± 0.01% after 5 and 20 min, respectively. After 5, 15, and 45 min of recovery, the corresponding values were 17.2 ± 0.07, 17.4 ± 0.07, and 17.8 ± 0.07%, respectively.

T_{CO_2} and also blood gas data were measured in experiments reported elsewhere (Folbergrová et al., 1985). Table 2 gives the T_{CO_2}, P_tCO_2, and tissue HCO_3^- content values, the last being calculated as T_{CO_2} minus the physically dissolved CO_2. Since the T_{CO_2} values were similar in the two control groups, the data were pooled. As observed, 5 and 20 min of seizures reduced the tissue HCO_3^- concentration to 50–60% of control. Although the number of experiments was too small to reveal any significant differences, the results suggest that the tissue HCO_3^- content in hypoxic animals was even lower. Notably, T_{CO_2} and tissue HCO_3^- content values rose to normal levels already after 5 min of recovery, with a tendency to increase further after 15 and 45 min of recovery.

The results demonstrate that pH_i decreased during seizures by a mean of 0.20 unit in normoxic animals and by 0.32 unit in hypoxic animals. The results suggest that some regulation of pH_i occurred in the 5- to 20-min period of seizures; and since this tendency was present also in the hypoxic animals, it occurred in spite of a rising tissue lactate content. In the normoxic and hypoxic series, recovery pH_i values exceeded controls from 5 min and onward. We note that this occurred in spite of P_tCO_2 values exceeding 45 mm Hg.

DISCUSSION

The present results give novel information on pH_e and pH_i during and following seizures. In discussing these results, we bring up inherent difficulties in the methods used to derive extra- and intracellular acid-base variables.

Extracellular acid-base changes

Although liquid ion-exchange pH electrodes with tip diameters of 1–3 μm should provide reliable and nontraumatic estimates of pH_e (Kraig et al., 1983; Mutch and Hansen, 1984), some problems of interpretation exist. One of these concerns the absolute pH values obtained. In the rat, cisternal CSF has a HCO_3^- concentration of ~27 μmol ml^{-1} at a P_{CO_2} slightly exceeding 40 mm Hg

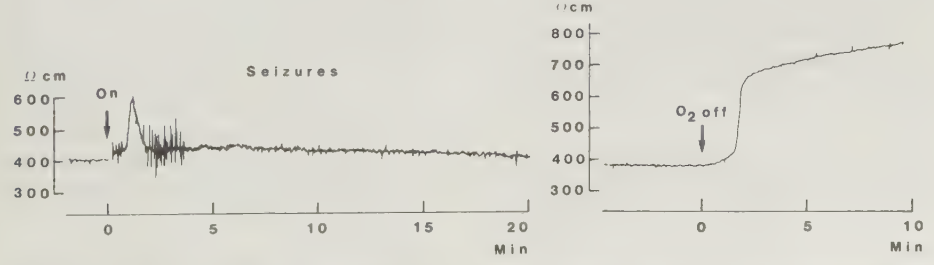

FIG. 6. Recording of impedance change during 20 min of seizures and during terminal anoxia. Note the small impedance change after 5 and 20 min of seizures. For further details, see the text.

TABLE 2. *Measured T_{CO_2} values and calculated values for P_tCO_2, $[HCO_3^-]_t$, $[HCO_3^-]_i$, and pH_i in normoxic and hypoxic animals exposed to 5 and 20 min of seizures, or allowed recovery periods of 5, 15, and 45 min*

Group	T_{CO_2} (μmol g^{-1})	P_tCO_2 (mm Hg)	$[HCO_3^-]_t$ (μmol g^{-1})	$[HCO_3^-]_i$ (μmol g^{-1})	pH_i
Control	11.88 ± 0.36	42.6 ± 0.56	10.64 ± 0.37	10.54 ± 0.66	7.01 ± 0.03
Seizures (N)					
5 min	6.95 ± 0.15	40.2 ± 0.98	4.63 ± 0.33	6.32 ± 0.18	6.81 ± 0.04[a]
20 min	7.85 ± 1.04	40.7 ± 2.24	4.73 ± 0.20	7.88 ± 1.46	6.89 ± 0.06
20 + 5 min	12.2 ± 0.98	43.2 ± 0.65	9.59 ± 0.49	14.57 ± 1.58	7.15 ± 0.05[a]
20 + 15 min	12.5 ± 0.96	42.7 ± 2.18	11.87 ± 0.46	14.57 ± 1.49	7.15 ± 0.04[a]
20 + 45 min	13.5 ± 0.91	45.7 ± 1.46	12.12 ± 0.88	14.95 ± 1.48	7.13 ± 0.03
Seizures (H)					
5 min	5.74 ± 0.36	38.1 ± 1.34	4.63 ± 0.33	4.55 ± 0.51	6.69 ± 0.04[b]
20 min	5.87 ± 0.21	39.2 ± 0.43	4.73 ± 0.20	5.47 ± 0.29	6.77 ± 0.02[b]
20 + 5 min	10.9 ± 0.53	45.3 ± 2.77	9.59 ± 0.49	12.2 ± 0.80	7.05 ± 0.03
20 + 15 min	13.2 ± 0.52	45.0 ± 1.97	11.87 ± 0.46	14.9 ± 0.65	7.14 ± 0.01[a]
20 + 45 min	14.0 ± 0.58	47.5 ± 1.82	12.62 ± 0.53	13.4 ± 0.70	7.07 ± 0.01

Values are means ± SEM (n = 4–5). Statistical differences were calculated for intracellular pH (pH_i) values only. T_{CO_2}, total tissue CO_2 content; P_tCO_2, tissue CO_2 tension; $[HCO_3^-]_t$, tissue HCO_3^- concentration; $[HCO_3^-]_i$, intracellular HCO_3^- concentration; N, normoxia; H, hypoxia.

[a] $p < 0.05$, [b] $p < 0.01$ by Newman Keul's test following analysis of variance.

(e.g., Messeter and Siesjö, 1971). Thus, the pH exceeds 7.4. The question arises as to why the pH_e is lower, by ~0.1 pH unit. As discussed by Mutch and Hansen (1984), this is either because P_{CO_2} is higher than usually assumed, or because the extracellular HCO_3^- content is lower than the CSF content. We agree with these authors that the latter possibility is more likely. Thus, calculation of P_tCO_2 from diffusion theory, direct tissue electrode measurements, and measurements of CSF P_{CO_2} all agree in showing that P_tCO_2 is lower than P_vCO_2 (Pontén and Siesjö, 1966; Brzezinski et al., 1967; Caronna et al., 1974). We submit, therefore, that the P_tCO_2 values derived presently are close to the true ones. It follows that the extracellular HCO_3^- content must be lower than the CSF content. Evidence to the contrary exists (Fencl et al., 1966). However, since three independent series (Kraig et al., 1983; Mutch and Hansen, 1984; and our own measurements) have consistently given control pH_e values of close to 7.3, we must, at least tentatively, conclude that the ECF proper is acidified by the influx of H^+ and efflux of HCO_3^-, perhaps by the constant transport of H^+ from nerve and/or glial cells. It should be emphasized, though, that the present conclusions regarding changes in pH_e and pH_i are not critically dependent on the absolute HCO_3^- values in the control state.

The pH_e changes recorded during seizures were similar to those recorded by others (e.g., Wang and Sonnenschein, 1955; Heuser, 1978). However, some findings are new. First, our results demonstrate that little or no "regulation" of pH_e occurs during the seizure period, since the 5- and 20-min values were identical. Second, the data clearly demonstrate an initial, fast acidification, resembling spreading depression. Third, evidence was obtained that pH_e, in contrast to pH_i, normalized at a strikingly slow rate upon cessation of seizure activity. We discuss these findings in turn.

Regulation of pH_e. It has often been assumed that pH_e is subject to regulation, either by H^+/HCO_3^- transport across the blood–brain barrier or by glial cells (see Leusen, 1972; Siesjö, 1972; Fencl, 1984). The present results suggest that such regulation is small or negligible during seizures, at least in the 20-min period studied. Possibly, this was due partly to the fall in arterial pH (and HCO_3^- concentration). Although the mechanisms of acidification are not precisely known, it seems probable that the efflux of lactic acid from cells is mainly responsible. Thus, if the nonionic diffusion of lactic acid predominates (see Roos and Boron, 1981), one can envisage that the amount of lactic acid "titrating" the ECF corresponds roughly to the increase in tissue lactate content (see MacMillan and Siesjö, 1971). After 5 and 20 min of seizure activity, the changes in lactate values were close to 10 μmol g^{-1} of tissue water (78% of tissue weight). In an isolated system with $[HCO_3^-]$ equal to 22 μmol g^{-1} and a pH of 7.31, this amount of lactic acid, combined with a P_{CO_2} increase of 4–5 mm Hg, should reduce the pH by ~0.3 unit. We note that this value is close to that measured. Tentatively, therefore, we conclude that ECF acidification at semi-steady state is caused primarily by the nonionic diffusion of lactic acid from the cells to the ECF.

Initial acid shift. Bicuculline-induced seizures raise tissue lactate content to 4–6 μmol g^{-1} during the first 30–60 s (Chapman et al., 1977). It was

therefore surprising that pH_e fell rapidly, at times by values exceeding 0.4 unit, during the first minute of fluorothyl seizures. The inescapable conclusion is that mechanisms other than lactic acid production must have contributed to this initial acidification. Probably, the explanation is that a spreading depression is elicited at seizure onset, as previously documented in bicuculline-induced seizures (Astrup et al., 1978). Spreading depression has previously been shown to be accompanied by an acid shift in pH_e of ~0.4 unit (Kraig et al., 1983; Mutch and Hansen, 1984). One probable mechanism is Na^+/H^+ exchange across cell membranes. Thus, if the seizures increase the Na^+ permeability of cell membranes, and if Na^+ influx occurs via a Na^+/H^+ antiporter, the ECF will be acidified. Clearly, such an exchange would curb and delay the intracellular acidosis caused by lactic acid accumulation. At first sight, the data of Heuser (1978) are contradictory. However, since this author used cats, the present results may reflect the fact that rats are more prone than other species to develop spreading depression [see also Harris et al. (1984) for data on hypoglycemia]. In contrast to previous measurements of pH_e at the onset of bicuculline-induced seizures (Astrup et al., 1978), the present results failed to reveal an initial alkaline shift in pH_e. In the previous study, electrodes with much larger tip diameters were used. This may have contributed to the differences in results (see Astrup et al., 1978). However, an even more probable explanation is that in the bicuculline experiments, P_aCO_2 fell rapidly at seizure onset, probably because peripheral vasoconstriction due to massive sympathoadrenal activation reduced the body mass perfused. In the present experiments, in which phentolamine was used to curb sympathetic vasoconstriction, the initial decrease in P_{CO_2} should have been small or absent.

Slow normalization. In contrast to hypercapnia, fluorothyl-induced seizures were followed by a strikingly slow normalization of pH_e, with relatively marked acidosis persisting after 15 min of recovery. Although this may have, at least in part, been due to the slow normalization of lactate content, it is noteworthy that pH_i showed fast normalization. It is conceivable that the regulation of pH_i by the extrusion of H^+ (see below) contributed to the persisting extracellular acidosis. At any rate, the results demonstrate that feeble mechanisms exist for the regulation of pH_e in the postepileptic situation. It is also worth mentioning that extracellular acidosis persists in the recovery period at times when the cerebral blood flow has decreased below control (G. Nevander and M. Ingvar, in preparation).

Intracellular acid-base changes

The degree of acidosis attained in normoxic animals after 5 min of seizure activity (~0.2 unit) is similar to that previously estimated with more indirect techniques (Howse et al., 1974; Duffy et al., 1975; Folbergrová et al., 1985). The present results suggest, though, that some regulation of pH_i occurs during ongoing seizures. The possibility also exists that some regulation occurred during the first 5 min. A system in which regulation by H^+/HCO_3^- transport does not occur, and which has the buffer capacity of cerebral intracellular fluids, can be expected to change its pH by ~0.35 unit when 10 $\mu mol\ ml^{-1}$ of strong acid is added (Siesjö, 1973, 1978). This is a somewhat larger pH change than that estimated for normoxic animals after 5 min. After 20 min of seizures, the reduction in pH_i for both normoxic and hypoxic animals was clearly lower than what would result from the accumulation of lactic acid. We conclude that the persistence of an adequate ATP source allows the extrusion of H^+ to occur.

A novel finding is that the arrest of seizure activity was followed by rapid normalization of pH_i and by postepileptic alkalosis. The results receive support from those obtained by the derivation of pH_i changes from the creatine kinase equilibrium (Folbergrová et al., 1985). Obviously, the results are akin to those obtained following ischemia, when postinsult alkalosis develops (Mabe et al., 1983). It seems reasonable to assume that the mechanisms are similar, that is, regulation of pH_i by acid extrusion, e.g., via the operation of a Na^+/H^+ antiporter, with the simultaneous removal of H^+ by oxidation of the lactate accumulated.

Based on the present results, we can derive a tentative scheme illustrating the events leading to intra- and extracellular acidification during seizures and to intracellular alkalosis in the recovery phase (Fig. 7). According to this scheme, the initial acid shift in pH_e is dominated by Na^+/H^+ exchange, and the sustained acidosis by the nonionic diffusion of lactic acid. Intracellularly, acidosis is a result of lactic acid production, the effect of which may be somewhat delayed and curbed by the Na^+/H^+ exchange. Upon the arrest of seizure activity, continued Na^+/H^+ exchange will, in conjunction with the (slow) oxidation of lactate and the removal of a stoichiometrical amount of H^+, give rise to the rapid normalization and subsequent increase of the pH above normal. Possibly, such acid extrusion delays the normalization of pH_e.

We wish to emphasize that although the changes in pH_i during and following seizures seem unequivocal, the results can give no hints as to whether the

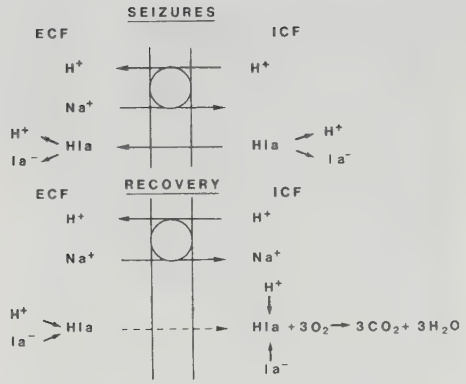

FIG. 7. Tentative scheme explaining the acidification of extracellular (ECF) and intracellular (ICF) fluids during seizures, and events occurring in the recovery period. For further explanation, see the text. Hla, lactic acid; la^-, lactate ions.

changes occur in neurons or glial cells or both, and they define no role for the Cl^-/HCO_3^- antiporter that seems to provide a means for ionic exchanges across glial cell membranes (see Kimelberg and Bourke, 1984). Elucidation of acid-base changes at the cellular level will require the use of other methods than those available at present.

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